Bulletin No. 26 December 1967

STUDIES ON THE PHYSIOLOGIC RACES OF THE RICE BLAST FUNGUS, *PIRICULARIA ORYZAE* CAV.

Chin-Chung Chien

Taiwan Agricultural Research Institute
Taipei, Taiwan, China

Bulletin No. 26 December 1967

STUDIES ON THE PHYSIOLOGIC RACES OF THE RICE BLAST FUNGUS, *PIRICULARIA ORYZAE* CAV.

Chin-Chung Chien

Taiwan Agricultural Research Institute
Taipei, Taiwan, China

Final Technical Report

Grant No.
FG-Ta-107

STUDIES ON THE PHYSIOLOGIC RACES OF THE RICE BLAST FUNGUS, *PIRICULARIA ORYZAE* CAV.

Chin-chung Chien

Department of Plant Pathology

Taiwan Agricultural Research Institute

Final report of the cooperative research project

FG-Ta-107

Supported

by

U.S.D.A.

July 1, 1964-June 30, 1967

Preface

The evidence of the presence of physiological races of rice blast fungus and its ralated problems in Taiwan have been a subject which attracted our interest. We have started studies on these problems in July 1964, under PL 480 (FG-Ta-107) research project entitled, "Studies on the physiologic race of the rice blast fungus, *Piricularia oryzae* Cav."

In this final report, we present the research results obtained from various experiments in the past three years, including identification and distribution of the blast fungal races in Taiwan, nutritional requirement of the blast fungus and genetical studies on the resistance of rice varieties to certain races of the blast fungus.

We are submitting this report to the United States Department of Agriculture with sincere thanks for the generous grant which has enabled us to carry out this research project.

Taipei
July 1967

Shoei-chyuan Hsu, D. Ag.
Director,
Taiwan Agricultural Research Institute

CONTENTS

Preface

Part I: Studies on the physiologic races of the rice blast fungus, *Piricularia oryzae* Cav ··· 1

Introduction ····· 1

Materials and Methods ····· 2

Experimental Results ····· 4

1. Identificatin of races of *P. oryzae* ····· 4
2. Prevalence and distribution of races of *P. oryzae* ····· 5
3. Reactions of the 16 Taiwan differential varieties to Japanese and U.S. races ·····11
4. Reactions of the U.S. and Japanese differentials to Taiwan races·····13
5. Reactions of the 12 tentative international differentials supplied by J. G. Atkins U.S.D.A. to Taiwan races of *P. oryzae*·····16
6. Reactions of the 24 tentative international differentials supplied by IRRI to Taiwan races of *P. oryzae*·····17
7. Reactions of 40 rice varieties supplied by FAO to Taiwan races of *P. oryzae*·····17
8. Reactions of commercial rice varieties to Taiwan races of *P. oryzae*·····17
9. Physiologic races of *P. oryzae* characterized in one field block·····21
10. Race characterization of *P. oryzae* by using the eight international differentials ·····22

Discussion and Conclusion·····24

Summary ·····26

Literature Cited ·····27

Part II: The vitamin, carbon and nitrogen nutrition of *Piricularia* oryzae Cav. ·····31

Introduction ·····31

Meterials and Methods·····32

Results ·····32

Effect of vitamins·····32

Effect of carbon sources·····38

Effect of nitrogen sources ·····38

Discussion ·····41

Summary ····43

Literature Cited····43

Part III: Inheritance of resistance to different races of *Piricularia oryzae* ····47

Introduction ····47

Materials and methods····47

Results

1. Reaction of F_2 seedlings to races 4 and 13····49
2. Relation between resistance gene Pi_4 and Pi_{13} ····52
3. Reaction of F_2 plants to races 22 and 25 ····53
4. Reaction of F_3 plants to races 4 and 22····55
5. Genotypes of blast resistance in parental varieties····56
6. Relation between leaf-and neck-blast resistances····57
7. Test of linkage between Pi_4;Pi_{22} and other marker genes ····58
8. Test of breeding materials with different races of the blast fungus····59

Discussion ····60

Summary ····61

Literature Cited ····61

Future plan of breeding for resistant varieties····62

PART I
STUDIES ON THE PHYSIOLOGIC RACES OF THE RICE BLAST FUNGUS, *PIRICULARIA ORYZAE* CAV.[1]

C. C. CHIEN[2], S. Y. LIN[2], C. L. CHU[3], T. G. CHOU[4] and K. C. LIU[2]

Introduction

Since 1922-23, Sasaki (24) first reported the variation of pathogenicity in rice blast fungus, *Piricularia oryzae* Cav., many workers (13, 14, 17, 23, 25, 31) have studied the problems of physiologic specialization of the fungus in Japan. Classification of the fungus into physiologic types by comparing physiologic characteristics of isolates, and clarification of possible correlation between culture types and pathogenicity of the fungus were attempted (29, 30). Hemmi (16) intended to classify the fungus into strains by their pathogenicity to leaf and ear of rice. Suzuki (26) classified the races of the blast fungal isolates according to germination type of conidia and formation of appressoria. A joint study on the race problem under the leadership of Goto (8, 9, 10) was initiated in 1955, with five prefectural agricultural experiment stations participating.

In the United States, studies of different varietal reactions to the rice blast in separate geographic areas of the U.S. resulted in the discovery of physiologic races of *P. oryzae*, and the existence of at least two races of the fungus was reported (20). In further study, Latterel *et al.* (19) characterized fifteen physiologic races by their disease reactions on ten rice varieties. In 1963, they reported that 25 physiologic races had been characterized from the isolates obtained from 17 countries and from 5 rice growing areas of the United States on the basis of the differential reactions of 12 varieties of rice (18). Atkins (1, 3) reported the prevalence and distribution of physiologic races of *P. oryzae* in the U. S. and added one new race to the 15 existing races.

Field evidence indicating possible existence of physiologic races in Taiwan was first obtainted by Hashioka (11), who tested eight varieties for blast infection in seven locations and found a greater difference in varietal reaction due to locations than to yearly fluctuation. Li (21) observed differences in varietal reaction in blast nurseries established at four locations in 1956. This observation stimulated further research activities on the race problem. The study on physiologic race of the blast fungus was started in Taiwan Agricultural Research Institute in 1957, and in Academia Sinica, 1960. Hung *et al.* (12), using

1. The authors are indebted to Drs. J. G. Atkins, C. R. Adair, F. M, Latterell, A. L. Robert, S. H. Ou, R. J. Chiu ,K. Goto and M. Yamada for their advice, and for providing cultures and rice seeds during the course of the investigation.

2. Associate Plant Pathologists.

3. Junior Plant Pathologist.

4. Research Assistant.

Department of Plant Pathology, Taiwan Agricultural Research Institute, Taipei, Taiwan, China.

16 differential rice varieties, recognized five pathogenic types among the seven isolates tested. Using the same set of differential varieties, tests were later made by Chien *et al.* with 67 isolates collected in 1960, 141 in 1961 and 146 in 1962. (6). They characterized these isolates into 19 physiologic races. These races were conveniently grouped into seven race groups characterized by their ability to infect certain groups of rice varieties. Successively six more races were identified by Chien and his co-workers (5). In Academia Sinica, another set of differentials was used for race characterization. In 1962, workers in Academia Sinica characterized 40 isolates into five physiologic races according to their virulence on the nine differentials (15).

Latterell *et al.* (18) suggested the need for superior differential varieties on an international basis and proposed 17 as possible candidates for a set of international differential varieties. In 1965 (4), a set of international differentials and nomenclature of races on an international basis by using the international differentials and standard techniques for uniform testing were proposed as the results of the United States-Japan Cooperative Rice Blast Project which was established in 1963.

In the course of cooperative efforts with the U.S.D.A., further characterization of races with isolates collected in Taiwan was made and the cultures of the blast races characterized by Chinese, Japanese and the U.S. workers were exchanged for investigation of their relationship. Several sets of differential rice varieties including U.S., Japanese differentials, 40 rice varieties from FAO, 24 tentative international differentials, 12 tentative international differentials, and 8 international differentials were tested as to their ability in differentiating isolates obtained in Taiwan, Japan and the U.S.

Materials and Methods

Isolation and maintenance of cultures

Isolations were made from the disease specimens collected during 1964-66 from the uniform blast nurseries. Pure cultures thus obtained were further subjected to single-spore transferring to yeast-starch agar slants. Stock cultures were maintained on rice straw medium in small test tubes and stored at 4° C.

Preparation of inoculum

Cultures to be tested for pathogenicity were first grown in 125 ml Erlenmeyer flasks containing 25 g water-soaked rice seeds and 10 ml water. After ten-day incubation at 28°C., the cultures were washed with sterilized water and removed to Petri dishes with their covers half open. Spores were produced abundantly in 3-6 days, depending on isolates. A second or third harvest of spores could be obtained by washing the cultures once or twice if they had not been contaminated.

Differential varieties

The 16 Taiwan differentials now in use for testing cultures of blast fungus and differentiating them into races are listed in Table 1. These include 11 varieties of the *japonica* type and five of the *indica* type. Of the *japonica* type, one was introduced from mainland China, three from Japan and seven were locally bred varieties. Of the *indica* type,

two are the upland rice and three the lowland rice (Table 1).

The U.S. differentials supplied by U.S. Army Biological Laboratory, Frederick, Maryland, the Japanese differentials by the Japan National Institute of Agricultural Science, the 40 rice varieties by FAO, the 24 tentative international differentials by IRRI and the 12 tentative international differentials by Dr. Atkins, U.S.D.A., the 8 international differentials by IRRI were used in the tests for their value in differentiating some representative Taiwan races of the blast fungus. The U.S. races kindly supplied by Dr. Atkins, and Japanese races by the Japan National Institute of Agricultural Science were used for testing their virulence to Taiwan differential varieties.

Table 1. Sixteen rice varieties employed at TARI as differentials for identification of physiologic races of *Piricularia oryzae* Cav.

Variety	Type	Origin
Kung-shan-wu-shen-ken	Japonica, lowland	Mainland, China
Taichung 65	〃	Taiwan
Pai-kan-tao	〃	〃
Taichung 171	〃	〃
Chianung 242	〃	〃
Kwang-fu 1	〃	〃
Chianung-yu 280	〃	〃
Taichung line 33	〃	〃
Kanto 51	〃	Japan
Norin 21	〃	〃
Sensho	〃	〃
Cutsugulcul	Indica, upland	Taiwan
Natala	〃	〃
Kao-chio-liu-chou	Indica, lowland	〃
Kaohsiung-ta-li-chen-yu	〃	〃
Taichung-ti-chio-wu-chien	〃	〃

Inoculation

The rice seeds sown for supplying test plants were treated with organic mercury fungicide and soaked in water for a few days at room temperature until they showed sign of germination. Plants were grown in paint-coated metal boxes (30cm×30cm×7.5cm in size), and placed on the greenhouse bench. Rice seedlings were top-dressed twice with 2% ammonium sulfate solution at the rate of 30 ml per box per application. Rice seedlings at the three to four-leaf stage were inoculated by spraying with spore suspension. Inoculated plants were incubated in a moist chamber of 20°-30°C for 24 hours before being removed to the greenhouse bench.

Recording of infection.

Five to seven days after inoculation, the inoculated plants were observed for reaction types which are classified as follows:

R (Resistant): Brown to dark brown small spots devoid of a grey center, usually 1 mm in diameter. The absence of lesion response also taken as R.

M (Moderately resistant): Brown to purple spots with grey center, about 2 mm in diameter.

S (Susceptible): Rapidly expanding lesions of medium to large size, usually with water-soaked appearance at first and a purplish brown border and a grey center later.

Experimental Results

1. Identification of races of P. oryzae Cav.

More than four hundred isolates of *P. oryzae* Cav. were obtained from the uniform blast nurseries in Ilan, Tungshu, Chiayi, Pingtung, Taitung, and Hualien areas during 1964-66, of which 293 isolates were tested on the 16 differential varieties and 19 races were characterized. Among them, 17 races showed the specialization pattern which had been previously detected (5). The addition of the two new races, races 26 and 27 increased the total number of races of the blast fungus to 27 races. These 27 races characterized during 1960-66 can be conveniently placed into seven race groups as follows:

Group A: Isolates of this group (race 1) were avirulent to the 16 differential varieties in greenhouse inoculation tests. They might have lost their pathogenicity during culturing, since most of them were originally isolated from leaf lesions of susceptible type.

Group K: Isolates of this race group (race 2 and 17) were characterized by their ability to infect KSWSK. In addition to their infectious ability to KSWSK, isolates of race 17 caused susceptible reaction on TC 65.

Group N: Isolates of this group (race 3) caused susceptible reaction on the *indica* variety Natala but resistant reaction on other differential varieties.

Group J: Four races (race 4, 5, 6, and 7) were included in this group. They were characterized by producing susceptible reactions on three *japonica type* varieties, (KT 51, NR 21 and SS). Reactions on other differential varieties were inconsistent.

Group I: This race group was composed of eight races (race 8, 9, 10, 11, 20, 21, 22, and 23). They were pathogenic to three local varieties of lowland *indica type* rice varieties.

Group P: Five races (race 12, 13, 14, 15, and 16) were placed under this group for their susceptible reaction on KSWSK and most of the locally developed *japonica* varieties. The variety Natala also gave susceptible reaction to the five races. Race 12 was the most virulent one among the 27 races so far characterized. It produced susceptible reaction on all 16 differential varieties.

Group T: Included in this race group were six races (race 18, 19, 24, 25, 26, and 27). This race group is characterized by its pathogenicity on KSWSK, TC 65 and

Natala.

The pattern of varietal reactions shown by the 27 races and the number of isolates obtained for each race during 1960–1966 are given in Table 2.

2. Prevalence and distribution of races of P. oryzae. Cav.

The distribution and frequency of occurrence of the 27 races of *P. oryzae* in Taiwan during 1964–66, together with those of 1960–63 are presented in Table 3 and Fig 1. Fig 2 gives the distribution of the blast races in Taiwan during 1960–66.

As shown in Table 3, races 24, 2 and 17 were the most predominant ones among 27 races during 1960–66. Race 13, the most predominant one during the years of 1960–63, and the major race in Tungshu and Chiayi areas, was not encountered during 1964–66, except one instance at Tungshu in 1966. On the other hand, only four isolates from four areas were designated as race 24 in 1963, however, it was encountered as many as 64 times during 1964–66. Races belonging to J group were not encountered in and after 1964. Races in P group, with the exception of race 12 which was encountered two times in 1964 and 3 times in 1965, of race 13 one time in 1966, and of race 16 one time in 1964, were not encountered in and after 1964. In I group, except race 9 which was characterized 3 times in 1965, and race 11, one time in 1964, all previously designated races before 1962 were not encountered in and after 1964. The rest of the races i. e., race 20, 21, 22 and 23 which were first designated in 1963 were encountered with steady frequencies during 1964–66.

There were fluctations in race appearances each year during the seven years period. The actual number of races detected was 13 races in 1960, 12 in 1961, 17 in 1962, 19 in 1963, 16 in 1964, 13 in 1965 and 14 in 1966.

In consideration of localities, 21 races were found in Ilan, 24 in Tungshu, 25 in Chiayi, 23 in Pintung, 25 in Taitung and 11 in Hualein. The distribution of the major races in the six areas were: Ilan, races 2, 14, 17 and 24; Tungshu, races 13 and 9; Chiayi, races 13, 17 and 2; Pintung, races 24, 17 and 2; Taitung, race 17 and Hualein, race 24. The patterns of race distribution in six localities where the uniform blast nurseries were located were quite similar. Among the 27 races so far characterized, 18 races were encountered over all the localities, except Hualein area, during the years of 1960–66.

Table 2. Physiologic races of *P. oryzae* characterized in Taiwan during 1960–1966.

Differential variety	Race designation																								Total no. of isolates	Total no. of races
	A	K		N	J				I								P					T						
	1	2	17	3	4	5	6	7	8	9	10	11	20	21	22	23	12	13	14	15	16	18	19	24	25	26	27	
Kung-shan-wu-snen-ken	R	S	S	R	S	S	S	S	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S		
Taichung 65	R	R	S	R	R	Sm	S	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S		
Pai-kan-tao	R	R	R	R	R	Mr	S	R	R	Rs	R	S	R	R	R	R	S	S	Sm	Sr	S	S	R	R	S	S	R		
Taichung 171	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S	R	S	R	S	R	S		
Chianung 242	R	R	R	R	R	R	S	R	S	R	R	R	R	R	S	S	S	S	S	S	S	R	S	S	R	S	S		
Kwang-fu 1	R	R	R	R	R	R	S	R	S	R	R	R	R	R	S	S	S	S	S	S	S	R	S	R	R	S	S		
Chianung-yu 280	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S	R	R	R	R	S	S		
Taichung-line 33	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S	R	R	R	R	R	S		
Kanto 51	R	R	R	R	S	M	S	S	R	R	R	R	R	R	R	S	S	R	Sr	R	S	R	S	R	R	R	R		
Norin 21	R	R	R	R	S	M	S	S	S	R	R	R	R	R	R	S	S	R	S	R	S	R	R	R	R	R	R		
Sensho	R	R	R	R	S	M	S	S	S	R	S	S	R	R	R	R	S	R	Sm	M	S	R	R	R	R	R	R		
Cutsugucul	R	R	R	R	Sm	S	S	S	R	R	R	S	R	S	S	S	Sm	S	Sr	R	S	R	R	R	R	R	S		
Natala	R	R	R	S	S	S	S	S	Sm	S	S	R	R	S	S	S	Sm	Sm	S	S	S	Sm	Sm	S	S	S	S		
Kao-chio-liu-chou	R	R	R	R	R	Sm	S	Sm	S	Sr	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R		
Kaohsing-ta-li-chen-yu	R	R	R	R	R	M	S	Sm	S	S	S	S	S	S	S	S	S	S	Sr	R	R	R	R	R	R	R	R		
Taichung-ti-chio-wu-chien	R	R	R	R	Sm	R	Sm	Sm	S	S	S	S	S	S	S	S	S	S	M	R	R	S	R	R	R	R	R		
1960~1963	60	36	59	6	15	6	5	8	4	21	23	3	5	4	8	2	29	55	30	16	12	27	8	4	4			450	25
1964	24	16	20	5								1	3	5	3		2				1	1	9	36	3	18	4	151	16
1965	2	6	4	2						3			2	5	3	2	3							11		5	1	49	13
1966	9	11	8	13									8	10	6	2		1					1	17	1	2	4	93	14
Total	95	69	91	26	15	6	5	8	4	24	23	4	18	24	20	6	34	56	30	16	13	28	18	68	8	25	9	743	27

Table 3. Distribution and frequency of occurrence of physiologic races of *P. oryzae* characterized in Taiwan during 1960–1966.

Source	Year	Number of isolates																								Total no. of isolates	Total no. of races
		Group																									
		A	K		N	J				I									P					T					
		Race																									
		1	2	17	3	4	5	6	7	8	9	10	11	20	21	22	23	12	13	14	15	16	18	19	24	25	26	27		
Ilan	1960	1	1	1														1	1	2				1					8	7
	1	2	1	1		4						1							2	12			1						24	8
	2	10	2	1	1			2	2		2		1						1				3						25	10
	3	1	3	5		1									1	1							1		1				14	8
	4	4	4	3	2										1										9				23	6
	5		1	1							1				1										1		1		6	6
	6	1	4	2	4									1		2	1								3				18	8
	Total	19	16	14	7	5		2	2		3	1	1	1	3	3	1	1	4	14			5	1	14		1		118	21
Tungshu	1960	3	1	1		2				2	3	2						4	11			1							30	10
	1	1	1	1		2					9	5						3	6	5			7						40	10
	2					1		1	1		2	1						1	1				2						10	8
	3	7		1	1									1	2	1	1		3			1				2			20	10
	4	5	1	2											3	1		2				1	1	1	1		1		19	11
	5		1		2											1	1								3		1		9	6
	6	1		3	4									2					1						2	1			14	7
	Total	17	4	8	7	5		1	1	2	14	8		3	5	3	2	10	22	5	1	2	10	1	6	3	2		142	24

| | | |

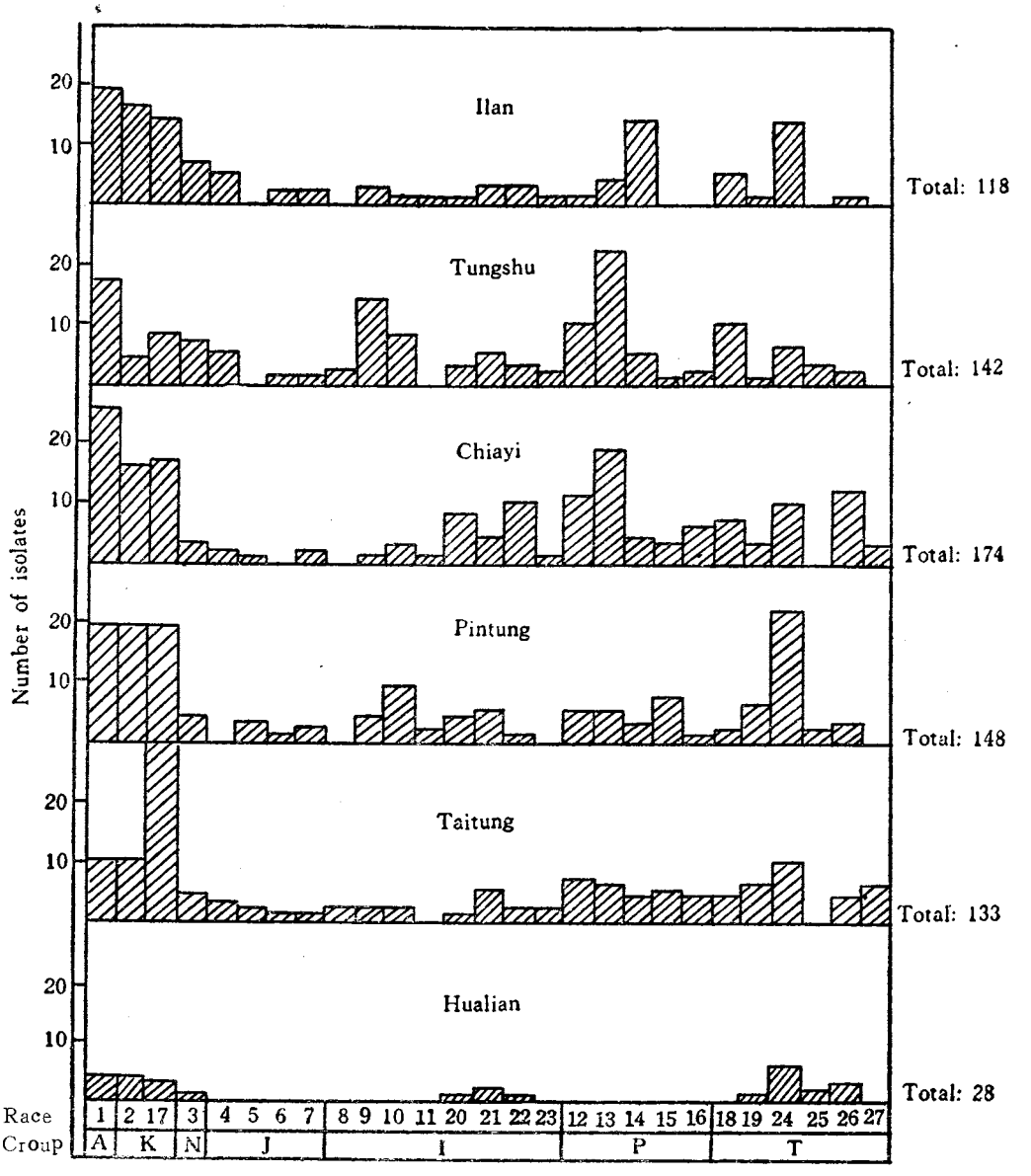
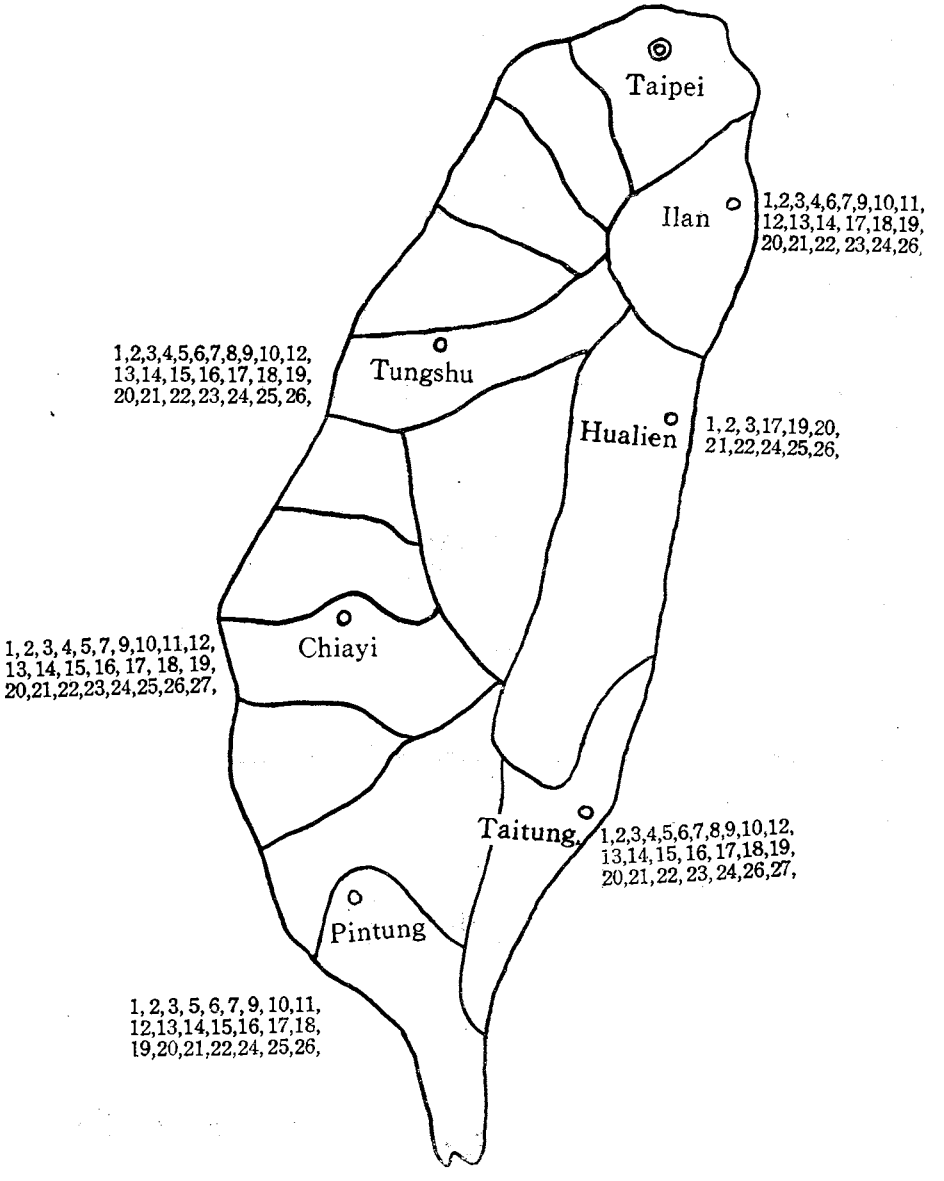


Fig 1. Distribution and frequency of occurrence of the 27 races of *P. oryzae* in Taiwan during 1960-66



Fig, 2. Geographic distribution of *Piricularia oryzae* races in Taiwan during 1960-1966

3. Reactions of the 16 Taiwan differential varieties to Japanese and U.S. races.

Eleven isolates representing eight Japanese races were tested for their pathogenicity on Taiwan differentials. The results are presented in Table 4.

Japanese race T-1 (Ken 53-33) could be included in P race group on Taiwan differentials. It infected all the 16 Taiwan differentials except CNY 280.

Japanese race T-2 did not show identical reactions for the two isolates (Ken 55-73 and Ken 62-88) when tested on the Taiwan differentials. Ken 55-73 gave a J race group reaction, while Ken 62-88 gave reaction similar to A race group, with susceptible lesions produced on KF-1 only.

Japanese race C-1 failed to give identical reactions for the two isolates (Ken 60-19 and ken 63-23) on the Taiwan differentials. Ken 60-19 gave the pathogenic pattern similar to Taiwan race 23. Ken 63-23 was less virulent than Ken 60-19.

Japanese race C-3 (Naga 87) gave a reaction similar to T race group on Taiwan differentials.

Japanese race N-1 (Hoku 373) gave the pathogenic pattern similar to Taiwan race 22 on Taiwan differentials. Taiwan race 22 gave the same reaction of N-1 on Japanese differentials as indicated in the later paragraph. It was believed that Japanese race N-1 was equavalent to Taiwan race 22.

Japanese race N-2 (Ai 62-22) gave the I race group reaction on Taiwan differentials.

Japanese race N-4 (Ina 168) behaved as Taiwan race 24 on Taiwan differentials. Yet Taiwan race 24 was not identical to Japanese N-4 on Japanese differentials.

Japanese race N-5 (Hoku 61-101) differed from any of the Taiwan races on Taiwan differentials. Naga 64-14, the other isolate in *Japanese race N*-5 gave the pathogenic reaction identical to Taiwan race 26 on Taiwan differentials. Yet Taiwan race 26 did not give the reaction pattern similar to the Japanese race N-5 on Japanese differentials.

Nine isolates representing six U.S. races were tested for their pathogenicity on the Taiwan differentials. The results are presented in Table 5.

U.S. Race 1, included two subraces, 1A and 1B. They were identical on the 16 differentials, if SM reactions were produced by race 1B on SS.

U.S. Race 3, produced SR and RS reactions respectively on KSWSK and TCTCWC. It gave resistant reaction on the rest of the varieties.

U.S. Race 4, caused RS reactions on KSWSK and KCLC, and R reaction on the rest of the varieties.

U.S. Race 5, included two subraces. Race 5A infected six varieties (KSWSK, NR 21, Nat., KCLC, KHTLC and TCTCWC), while race 5B infected five varieties (KSWSK, CNY 280, KCLC, KHTLC, and TCTCWC) of the Taiwan differentials.

U.S. Race 6, included two subraces, 6A and 6B. They were identical if RS reactions are produced on Cats by race 6B.

U.S. Race 7, caused S reactions on KSWSK, and RS reactions on three other varieties (KT 51, Nat., and KCLC).

Among the 16 Taiwan differntials, *indica type* varieties seemed more valuable as differentials than the *japonica type* varieties. Five *japonica type* varieties, i.e., TC 65 PKT, TC 171, CN 242, and KF 1 are not susceptible to any of the six U.S. races. Sucespitible reactions were produced on one *Japonica* type variety KSWSK by all the races.

Table 4. Reactions of Taiwan differential varieties to Japanese races of *P. oryzae*.

Variety	Japanese race										
	T-1	T-2	T-2	C-1	C-1	C-3	N-1	N-2	N-4	N-5	N-5
	Ken 53-33	Ken 55-73	Ken 62-88	Ken 60-19	Ken 63-23	Naga 87	Hoku 373	Ai 62-22	Ina 168	Hoku 61-101	Naga 61-14
Kung-shan-wu-shen-ken	S	S	R	S	S	S	S	S	S	S	S
Taichung 65	S	S	R	S	R	S	S	S	S	S	S
Pai-kan-tao	Sr	R	R	R	R	R	R	R	R	R	R
Taichung 171	Sr	R	R	S	R	R	S	R	R	Sr	S
Chianung 242	Sr	Sr	R	S	R	S	S	R	S	R	S
Kwang-fu 1	Sr	R	Sr	S	Sr	R	S	R	R	Sr	S
Chianung-yu 280	R	R	R	Sr	R	R	S	R	R	Sr	S
Taichung-line 33	Sr	R	R	S	R	R	S	R	R	R	R
Kanto 51	Sr	R	R	S	S	S	R	R	R	Sr	Sr
Norin 21	Sr	R	R	Mr	R	R	M	Sm	R	R	R
Sensho	Mr	R	R	R	R	R	R	Sr	R	R	R
Cutsugucul	S	R	R	S	Sr	R	S	R	R	R	R
Natala	S	R	R	S	Sr	S	S	S	S	R	S
Kao-chio-liu-chou	Mr	S	R	Sr	Sr	R	M	S	R	R	R
Kaohsing-ta-li-chen-yu	S	S	R	Sr	R	R	Ms	S	R	R	R
Taichung-ti-chio-wu-chien	S	S	R	Sr	R	R	Ms	S	R	R	R
Taiwan race equivalent				Ta.23			Ta.22?		Ta.24		Ta.26

Table 5. Reactions of Taiwan differential varieties to U.S. races of *P. oryzae*

Variety	U.S. race								
	1 A	1 B	3	4	5 A	5 B	6 A	6 B	7 A
Kung-shan-wu-shen-ken	S	S	SR	RS	S	S	S	S	S
Taichung 65	R	R	R	R	R	R	R	R	R
Pai-kan-tao	R	R	R	R	R	R	R	R	R
Taichung 171	R	R	R	R	R	R	R	R	R
Chianung 242	R	R	R	R	R	R	R	R	R
Kwang-fu 1	R	R	R	R	R	R	R	R	R
Chianung-yu 280	R	R	R	R	R	M	R	R	R
Taichung-line 33	R	R	R	R	S	R	R	R	R
Kanto 51	R	R	R	R	R	R	R	R	RS
Norin 21	R	R	R	R	R	RS	R	R	R
Sensho	SM	R	R	R	R	R	R	R	R
Cutsugucul	R	R	R	R	R	R	RS	R	R
Natala	R	R	R	R	S	R	S	S	RS
Kao-chio-liu-chou	S	S	R	RS	S	S	S	S	RS
Kaohsing-ta-li-chen-yu	S	S	R	R	S	S	S	S	R
Taichung-ti-chio-wu-chien	S	S	RS	R	S	S	S	S	R

4. Reactions of the U.S. and Japanese differentials to Taiwan races

Table 6 gives the reactions of the Japanese and U.S. differentials, respectively, to representative isolates of certain Taiwan races of *P. oryzae*. Most of these isolates were sent to the United States and Japan for tests at Beltsville, Maryland and Tokyo in exchange of cultures for the cooperative research.

It seemed that the U.S. differentials did not provide a good differentiation for the Taiwan races. Races 9, 22 and 24 were indistinguishable on the U.S. differentials. The same reactions were also obtained for races 13 and 16, and for races 14, 17 and 19. But, rather satisfactory separation of these races was obtained with Japanese differentials.

Taiwan race 1 (3C-58G) lost their pathogenicity during culturing. None of the Taiwan, U.S. or Japanese differentials were infected by this isolate.

Taiwan race 2 (2T-74S) gave susceptible reaction only on KSWSK of the 16 Taiwan differentials. It infected CI 8970-P and Aichi-asahi of the U.S. and Japanese differentials, respectively.

Taiwan race 3 (2K-27S) infecting only Natala of the Taiwan differentials induced susceptible reactions on Rexoro and Taichung 65 of the U.S. differentials. However, Taich-

ung 65 when tested along with other Taiwan varieties showed resistant reaction to this isolate. Usen and Ishikari-shiroke in the Japanese differentials were found susceptible to this race.

Taiwan race 5 (2K-24S) belonging to J race group on Taiwan differentials was pathogenic to five varieties of the U.S. differentials, namely, Rexoro, Lacrose, Caloro, CI 8970-P and Taichung 65. It showed the same pathogenic pattern as U.S. race 6. However, U.S. race 6 did not give the Taiwan race 5 pattern on the Taiwan differentials. On the Japanese differentials, it was pathogenic to Usen, Ishikari-shiroke, Homare-nishiki, Aichi-asahi, Norin 20 and Pi No. 1.

Taiwan race 9 (3C-109M) seemed to be different from any of U.S. and Japanese races. It was highly pathogenic to the Japanese differentials. Of the U.S. differentials, only Caloro, CI 8970-P and Taichung 65 were found susceptible to this isolate.

Taiwan race 12 (2S-74S) infected all of the Taiwan differentials. On the U.S. differentials, it induced the pathogenic pattern similar to U.S. race 21. However, U.S. 21 did not show Taiwan race 12 pattern on the Taiwan differentials. It gave the same pathogenic pattern as Japanese race N-6, on the Japanese differentials.

Taiwan race 13 (2C-2124S) showed the highest virulence to both the U.S. and Japanese differentials. Te-tep, Tudukan, Chokoto and Yakeiko were the only varieties among the Japanese differentials which were resistant to this race. It differed from any of the Japanese races on the Japanese differentials. When tested on the U.S. differentials, Taiwan race 13 gave the reaction pattern identical to U.S. 17, yet U.S. rece 17 did not show the Taiwan race 13 pattern on the Taiwan differentials.

Taiwan race 14 (1I-29S) representing one of the most virulent races on the Taiwan differentials showed only moderate pathogenicity on the Japanese differentials and weak pathogenicity on the U.S. differentials. This isolate induced the pathogenic pattern similar to U.S. race 4 on the U.S. differentials. On the Japanese differentials, it showed the pathogenic pattern similar to Japanese N-2, if susceptible reaction was not obtained on Pi No. 1.

Taiwan race 16 (2C-86S) gave the same pathogenic pattern as U.S. race 17 on the U.S. differentials. It seemed to be different from any of the Japanese races.

Taiwan race 17 (2K-82S) gave the same pattern of Japanese N-2 on Japanese differentials. It behaved like U.S. race 2 on the U.S. differentials.

Taiwan race 19 (4K-18S) showed weak pathogenicity to both the U.S. and Japanese differentials. It gave susceptible reactions on Caloro and Taichung 65 in the U.S. differentials. Of the Japanese differentials, only Norin 20 and Norin 22 were infected.

Taiwan race 22 (4T-28S) showed rather strong pathogenicity to the Japanese differentials, but weak pathogenicity to the U.S. differentials. The pathogenic pattern of this race on the Japanese differentials was identical to the Japanese race N-1.

Taiwan race 24 (4C-25S) produced the same pathogenic pattern as Taiwan races 9 and 22 on the U.S. differentials.

Taiwan race 26 (4T-27Sb) seemed to be different form any of U.S. and Japanese races. It gave the pathogenic patterns similar to Taiwan races 9, 22 and 24, on the U.S. differentials.

Table 6. Reactions of U.S. and Japanese differential varieties to Taiwan races of *P. oryzae*.

Variety	Taiwau Race													
	1	2	3	5	9	12	13	14	16	17	19	22	24	26
	3C-58G	2T-74S	2K-27S	2K-24S	3C-102M	2S-74S	2C-2124S	1I-29S	2C-86S	2K-82S	4K-18S	4T-28S	4C-28S	4T-27Sb
U.S. differentials														
Zenith	R	R	R	R	R	R	S	R	S	R	R	R	R	R
Rexoro	R	R	Sr	S	R	S	S	R	S	R	R	R	R	R
Lacrosse	R	R	R	S	R	R	S	R	S	Rs	R	R	R	R
Caloro	R	R	R	S	S	S	S	S	S	S	S	S	S	S
CI 8970-P	R	S	R	S	S	S	S	R	S	R	R	S	S	S
CI 8970-S														
CI 5309	R	R	R	R	R	R	R	R	R	R	R	R	R	R
PI 180061	R	R	R	R	R	R	R	R	R	R	R	R	R	M
PI 201902	R	R	R	R	R	R	R	R	R	R	R	R	R	R
PI 231128	R	R	R	R	R	R	R	R	R	R	R	R	R	R
PI 231129	R	R	R	R	R	R	R	R	R	R	R	R	R	Sr
Taichung 65	R	R	Rs	S	S	S	S	S	S	S	Sr	Sr	S	M
Japanese differentials														
Te-tep	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Tadukan	R	R	R	R	R	S	R	R	R	R	R	R	R	R
Usen	R	R	S	S	S	R	S	Rs	S	R	R	R	R	R
Chokoto	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Yakeiko	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kanto No.51	R	R	R	R	R	R	S	R	R	R	R	R	R	R
Ishikari Shiroke	R	R	S	S	S	S	S	R	S	R	R	S	S	S
Homare-nishiki	R	R	R	S	R	R	S	Rs	Rs	S	R	S	R	R
Ginga	R	R	R	R	S	R	S	S	Rs	S	R	S	S	S
Norin No.22	R	R	R	R	S	S	S	S	Rs	S	S	S	S	S
Aichi asahi	R	S	R	S	S	S	S	S	S	S	R	S	R	S
Norin No.20	R	R	R	S	S	S	S	S	S	S	S	S	R	S
Pi. No. 1	R	R	R	S	M	R	S	S	S	R	R	M	R	S
U.S. race equivalent	U.S. 10?			U.S. 6?		U.S. 21?	U.S. 17?	U.S. 4?	U.S. 17?	U.S. 2?				
Japanese race equivalent						N.6				N.2		N.1		

5. Reactions of the 12 tentative internat ional differentials supplied by J. G. Atkins, U.S.D.A. to Taiwan races of P. oryzae.

All varieties in groups 1 (Tadukan), 2 (Zenith and NP. 125), 3 (Usen), and 4 (C. I. 5309, Kanto 51, and Dular) gave resistant reactions to the eight races tested, with one exception that RS reaction was induced by race 16 on Usen, the only variety in group 3. On C.I. 8970-S, the variety in group 5, resistant reaction was caused by race 1, while susceptible reaction by races 16, 17, 22, and 26. SR or RS reactions were produced by races 3, 5, and 11 on this variety. Three varieties (Norin No. 20, Caloro, and Aichi asahi) in group 6 reacted resistantly to races 1 and 11 but susceptiblely to rece 22. Inconsistent reactions were shown on this variety group by the rest of the races. (Table 7)

Table 7. Reactions of the tentative international differentials supplied by J. G. Atkins. U.S.D.A. to Taiwan races of *P. oryzae*

Group no. and variety	Taiwan race							
	1	3	5	11	16	17	22	26
	3C-58G	2K-27S	2K-24S	2K-20S	2C-86S	2K-82S	4T-28S	4T-27Sa
Group 1								
Tadukan	R	R	R	R	R	R	R	R
Group 2								
Zenith	R	R	R	R	R	R	R	R
NP. 125	R	R	R	R	R	R	R	R
Raminad str.3								
Group 3								
Usen	R	R	R	R	R S	R	R	R
Group 4								
C.I. 5309	R	R	R	R	R	R	R	R
Kanto No. 51	R	R	R	R	R	R	R	R
Dular	R	R	R	R	R	R	R	R
Group 5								
C.I. 8970-S	R	S R	S R	R S	S	S	S	S
Group 6								
Norin No. 20	R	R	R	R	R	R S	S	S
Caloro	R	S R	S R	R	S	S R	S	S
Aichi asahi	R	R	R S	R	S	R	S	R

6. Reactions of the 24 tentative international differentials suupplied by IRRI to Taiwan races of P. oryzae.

Ten isolates representing ten races were tested for their pathogenicity to the 24 tentative international differentials. No pathogenic reactions were observed in all races on varieties of groups 1 (Te-tep and Tadukan), 2 (Zenith, Wag-wag, Raminad str. 3 and NP-125) and 4 (Chokoto, Yakeiko, G.I. 5309, Kanto 51 and Dular). Races 3, 13, 16, and 22 caused S-R reactions on varieties of group 3, while resistant reactions were given by races 1, 5, 11, 14, 17, and 26 on varieties of this group (Usen, Pi. No. 1, and Lacrosse). Varieties of group 5 (Ishikari-Shiroke, C. I. 8970-P, C. I. 8970-S, and Rexoro) gave resistant reaction to races 1, 5, 11, and 13. Only one or two varieties of this group gave RS or SR reaction to races 3, 17, 22, and 26. Race 16 is pathogenic to three varieties of this group. All varieties in Group 6 (Homare nishiki, Ginga, Taichung 65, Norin No. 22, Caloro, and Aichi asahi) reacted resistantly to race 1, but susceptiblely to race 22. Races 11, 16, 17, and 26 infected two varieties (Taichung 65 and Caloro) of the group, while races 3, 5, and 13 infected three varieties. (Table 8).

7. Reactions of 40 rice varieties supplied by FAO to Taiwan race of P. oryzae.

The 40 rice varieties from FAO were tested for their reactions to nine Taiwan races of *P. oryzae*. The results are presented in Table 9. All nine races caused resistant reaction on varieties of group 1, but susceptible reactions on varieties of group 6. Moderately resistant reactions were produced by races 12, 13, 17 and 22 on only one or two varieties of group 2. Varieties of group 3 seemed highly resistant to the races tested. Races 14, 17 and 18 all produced resistant reactions on this variety group; races 5, 13, and 22 each induced susceptible reaction only on one variety; races 9 and 12 induced susceptible reactions on two varieties among 8 varieties. Susceptible reactions were produced by race 3 on three varieties in the same group. Varieties of group 4 gave resistant reactions to races 3 and 13. Only one variety of this group gave susceptible reactions to races 5 or 18. Races 9 and 14 were pathogenic to two varieties of this group. Racec 12, 17 and 22 caused S-M reaction on four varieties of the group. Resistant reactions were produced by race 14 on all varieties of group 5. Race 17 and 18 caused MR and SR reactions respectively on Woo-Gen and T 141. The rest of the races are highly pathogenic to the varieties of this group.

8. Reactions of commercial rice varieties to Taiwan races of P. oryzae.

Twenty commercial rice varieties including two native Taiwan varieties i.e., Ai-Chueh-Chien and Taichung-native 1, and 18 *japonica* type varieties were tested for their reactions to eight Taiwan races of *P. oryzae*. Results are given in Table 10. Races 12, 13, and 16 are of the most pathogenic *ones* among the races tested. Of 20 varieties tested, only Taichung 186 Taichung-yu 184 were resistant to these races, while Taichung-native 1 resistant to races 12 and 13, but susceptible to race 16. Races 17, 24, and 26, the races of second pathogenic caused resistant reactions on the varieties of group 1 (Taichung 186 and Taichung-yu 184), but susceptible reactions on all varieties of group 2 (Taichung 65, Kaohsiung 24, Taipei 306 and Taipei 308) and group 3 (Tainan 1, Chianan 8, Kaohsinng 10, Kaohsiung 27, Kaohsiung

64, and Hsinchu 56). These three races induced S reaction on one or two varieties in group 4, and S-M-R reactions on some varieties of groups 5. Race 5 caused susceptible reactions on all varieties in group 5 (Taichung 178, Kaohsiung 122, Ai-Chueh-Chian, Taichung-native 1), and on two varieties in group 4 (Chianung 242 and Taipei 177). All varieties in groups 1 and 3 and two varieties in group 4 gave resistant reactions to the race. Race 1, the weakest in pathogenicity, caused resistant reactions on most of the verieties, a few varieties (Kaohsiung 24, Chianan 8, Kaohsiung 10, 64 and Hsinchu 56), however, showed RS reactions to the race.

Table 8. Reactions of the 24 tentative international differentials supplied by IRRI to Taiwan races of *P. oryzae*.

Group no. and variety	Taiwan race									
	1	3	5	11	13	14	16	17	22	26
	3C-58G	2K-27S	2K-24S	2K-20S	2C-2124S	1I-29S	2C-86S	2K-82S	4T-28S	4T-27Sa
Group 1										
Te-tep	R	R	R	R	R	R	R	R	R	R
Tadukan	R	R	R	R	R	R	R	R	R	R
Group 2										
Zenith	R	R	R	R	R	R	R	R	R	R
Wag-wag	R	R	R	R	R	R	R	R	R	R
Raminad str. 3	R	R	R	R	R	R	R	R	R	R
NP-125	R	R	R	R	R	R	R	R	R	R
Group 3										
Usen	R	RS	R	R	RS	R	S	R	SR	R
Pi. No. 1	R	RS	R	R	RS	R	SR	R	R	R
Lacrosse	R	R	R	R	RS	R	S	R	SR	R
Group 4										
Chokoto	R	R	R	R	R	R	R	R	R	R
Yakeiko	R	R	R	R	R	R	R	R	R	R
C. I. 5309	R	R	R	R	R	R	R	R	R	R
Kanto 51	R	R	R	R	R	R	R	R	R	R
Dular	R	R	R	R	R	R	R	R	R	R
Group 5										
Ishikari-shiroke	R	R	R	R	R	R	R	R	R	R
C. I. 9870-P	R	R	R	R	R	RS	S	RS	SR	R
C. I. 8970-G	R	RS	R	R	R	RS	S	R	S	RS
Rexoro	R	R	R	R	R	R	R	R	R	R

Group 6										
Homare-nishiki	R	R	RS	R	R	RS	R	R	S	R
Ginga	R	R	RS	R	R	RS	R	R	SR	R
Taichung 65	R	RS	SR	RS	S	S	S	S	S	S
Norin 22	R	R	R	R	R	S	R	R	S	R
Caloro	R	SR	SR	S	S	S	S	S	S	S
Aichi-asahi	R	SR	R	R	S	S	R	R	S	R

Table 9. Reactions of the 40 rice varieties supplied by FAO to Taiwan races of *P. oryzae*.

Group no. and variety	Taiwan race								
	3	5	9	12	13	14	17	18	22
	2K-27S	2K-24S	3C-102M	2S-74S	2C-2124S	1I-29S	2K-82S	2S-17S	4T-28S
Group 1									
PI 180061	R	R	R	R	R	R	R	R	R
Kanto 51	R	R	R	R	R	R	R	R	R
M-302	R	R	R	R	R	R	R	R	R
H-4	R	R	R	R	R	R	R	R	R
H-5	R	R	R	R	R	R	R	R	R
Murungakayan 302	R	R	R	R	R	R	R	R	R
Slo 15 (from 61A)	R	R	R	R	R	R	R	R	R
BR 7	R	R	R	R	R	R	R	R	R
Ninidhan	R	R	R	R	R	R	R	R	R
221/BCIV/I/178/3	R	R	R	R	R	R	R	R	R
221/BCIV/I/178/11	R	R	R	R	R	R	R	R	R
Hakkodo	R	R	R	R	R	R	R	R	R
G. B. 121	R	R	R	R	R	R	R	R	R
H-105	R	R	R	R	R	R	R	R	R
Samo Ran	R		R	R	R	R	R	R	R
Group 2									
Samo Trang	R	R	R	R	R	R	RM	R	R
FE 165	R	R	R	R	M	R	R	R	R
Tadukan	R	R	R	RM	M	R	R	R	R
Hybrid I	R	R	R	MR	R	R	R	R	M

Group 3									
Dud Kuning	R	R	S R		R	R	R	R	
Yakeiko	R	R	S R	R	R	R	R	R	R
Bengawan	S	R	R	R	R	R	R	R	R
D 25-4	S	R	R	R	R	R	R	R	R
Nepali Tulasi (Sel)	R	R	R	S	R	R	R	R	R
C 46-15	R	R	R	R	S	R	R	R	R
Hybrid II	R	R	R	S R	R	R	R	R	S
Fa-Yiu Tsai	S	S	R	R	R	R	R	R	R
Group 4									
A. C. 2250	R	R	R	RM	R	R	S R	R	S
Ginga	R	R	R	M	R	S	R	R	S
Nhta-6	R	R	S	S	R	R	RM	R	R
C 33-18	R	R	R	R	R	R	S R	S R	S R
T.K.M. 6	R	S R	S	R	R	R	R	R	S R
G.S. 336	R	R	R	S	R	R	S R	R	R
Group 5									
Tjere Mas	M	S R	M	R	R	R	R	R	RM
Woo-Gen	R	R	S R	S	R	R	MR	R	S
Patnai 23	S	S R	R	R	S	R	R	R	M
T 141	S	S	S R	R	S	R	R	S R	RM
M.T.U. 3	S	S	S	S	S	R	R	R	R
Group 6									
Usen	S	S	S R	S	S	S	S M	S	S
U.S.A. Caloro	S	S M	S	S	S	S	S R	S	S

Table 10. Reactions of commercial rice varieties to Taiwan races of *P. oryzae*.

Group no. and variety	Taiwan race							
	1	5	12	13	16	17	24	26
	3C-58G	2K-24S	2S-74S	2C-2124S	2C-86S	2K-82S	4C-25S	4T-27Sa
Group 1								
Taichung 186	R	R	R	R	R	R	R	R
Taichung-yu 184	R	R	R	R	R	R	R	R

Group 2								
Taichung 65	R	R	S	S	S	S	S	S
Kaohsiung 24	RS(17:3)[a]	S	S	S	S	S	S	S
Taipei 306	R	S	S	S	S	S	S	S
Taipei 398	R	S	S	S	S	S	S	S
Group 3								
Tainan 1	R	R	S	S	S	S	S	S
Chianan 8	RS(18:2)	R	S	S	S	S	S	S
Kaohsiung 10	RS(18:2)	R	S	S	S	S	S	S
Kaohsiung 27	R	R	S	S	S	S	S	S
Kaohsiung 64	RS(17:3)	R	S	S	S	S	S	S
Hsinchu 56	RS(16:4)	R	S	S	S	S	S	S
Group 4								
Chianung 242	R	S	S	S	S	R	S	S
Taipei 177	R	S	S	S	S	S	R	S
Tainan 3	R	R	S	S	S	S	R	R
Hsinchu 61	R	R	S	S	S	R	R	R
Group 5								
Taichung 178	R	S	S	S	S	RM(16:4)	S	RS(15:4)
Kaohsiung 122	R	S	SR(13:7)	S	S	MR(12:8)	S	RM(17:3)
Ai-chueh-chien	R	S	RS(13:7)	S	S	RS(15:5)	R	R
Taichung-native 1	R	RS(13:7)	R	R	S	R	RM(10:7)	R

a. The number of leaves for different reactions.

9. **Physiologic races of P. oryzae characterized in one field block**

Thirty five isolates of *P. oryzae* were isolated from the disease specimens collected randomly from a blast nursery consisting of one rice variety Taichung 65. Cultures of *P. oryzae* were single-spore isolated and tested for their pathogenicity on the 16 differential varieties in the greenhouse.

The results presented in Table 11 indicate that there are several races of *P. oryzae* existing in one field block. The difference in frequency of occurrence was shown by the races. Fourteen isolates were identified as race 26, seven isolates as race 24, three isolates as race 21 and the other three isolates as race 1. Only one isolate was identified as race 20. There were 5 isolates showing the pathogenicity which did not belong to the existing race patterns.

These races can be further placed into four race groups. Race 1 belonged to A race group, race 17 to K race group, race 20 and 21 to I race group, and race 24 and 26 to T

race group. The rest of isolates comprising 14.3% of the total isolates did not belong to any race group. It was noticed from this experiment that the most prevalent race belonged to the T race group. No isolates were identified as races belonging to J or P race group, although the disease specimens were collected from *japonica* type rice variety.

Table 11. Physiologic races of *P. oryzae* Cav. characterized in one field block

Group	A	K	I		T		Isolates not included in the existing race
Race	1	17	20	21	24	26	
Number of isolate	3	2	1	3	7	14	5
Percentage of isolate in race group	8.5	5.8	11.4		60.0		14.3

10. Race characterization of P. oryzae Cav. by using the eight international differentials.

The eight international differential varieties were tested for their reactions to the isolates of *P. oryzae* which had been characterized with the U. S., Japanese or Taiwan set of differential varieties, and to some newly collected isolates of *P. oryzae* in Taiwan. The results are given in Table 12.

when the isolates of Japanese races were characterized with the international set of differentials, five former Japanese races T-1, T-2, C-1, C-3, N-1, would fall into IC-5, ID-10, IE-1, IF-2, IG-1 respectively. Former Japanese races N-2, N-4. and N-5 would be included in IH-1. Former U.S. races 1, 7, 3, 5B would fall into IB-5, IF-1, IG-1 and IG-2 respectively. Former U.S. raes 5A and 6B, and races 2 and 4 would be designated as ID-8 and IH-1 respectively. Former Taiwan races 3, 4, and 5 could be designated as ID-8, race 16 as IE-1, races 9, 12, 13, 20, 22, 24, 26, and 27 as IG-1, race 21 as IG-2, and races 2, 17, and 19 as IH-1. However, in the U.S.-Japanese Cooperative Study, former Japanese races T-1, and N-5 were designated as IC-1 and IG-1 respectively, and former U. S. races 5A, 6B and 7A were designated as ID-9, ID-8, and IB-2 or IB-4, respectively (4).

When, newly collected isolates in 1966 were tested on the international set of differentials, almost three fourths of the isolates were characterized into IG and IH race groups. Since the key varieties of these group, i.e., C.I. 8970 (S) and Caloro are susceptible to most races (4), the Taiwan isolates designated as IG or IH groups could be considered as the races of rather weak pathogenicity.

It was also noticed that the strains of *P. oryzae* designated by using the international differentials could be split into more different race groups when the Taiwan differential varieties were used for race characterization. For instance, isolates designated as IG-1 could be split into eight Taiwn races included in P, I, and T race groups.

Table 12. Pathogenic races and isolates characterized by the 8 international differential varieties

International race group	International race	Reaction per differential variety								Prior race			newly collected Taiwan isolate
		Raminad Str. 3	Zenith	NP-125	Usen	Dular	Kanto 51	CI. 8970 (S)	Caloro	US	Japan	Taiwan	
IB	5	R	S	R	R	S	R	S	R	1A			
IC	3	R	R	S	R	S	S	S	S				1
	5	R	R	S	R	R	S	S	S		T-1 (IC-1)		
ID	8	R	R	R	S	R	R	S	S	5A (ID-9)[a] 6B (ID-8)		3,4,5	2
	10	R	R	R	S	R	R	R	S		T-2		1
	11	R	R	R	S	R	R	R	R				
IE	1	R	R	R	R	S	S	S	S		C-1	16	3
	2	R	R	R	R	S	S	R	S				1
	3	R	R	R	R	S	R	S	S				3
	4	R	R	R	R	S	R	R	S				1
IF	1	R	R	R	R	R	S	S	S	7A(IB-2, IB-4)			4
	2	R	R	R	R	R	S	S	S		C-3		
IG	1	R	R	R	R	R	R	S	S	3	N-1	9,12,13,20, 22,24,26,27	24
	2	R	R	R	R	R	R	S	R	5B		21	15
IH	1	R	R	R	R	R	R	R	S	2,4	N-2 N-4 N-5 (IG-1)	2,17,19	14

a. The race designated by the U.S.-Japanese Cooperative Study.

Discussion and Conclusion

The prevailing races of the blast fungus varied from year to year. Among the 27 races so far characterized, races 17, 2 and 24 were the most predominant ones. Race 13, the most predominant one of 1960-1961, became less prevalent later in 1962 and 1963, and was not encountered during 1964-1965. Only one isolate collected from Tungshu was characterized as race 13 in 1966. The same trend was also observed with race 12. Races in J and P groups with a few exceptions, were not encountered in and after 1964. On the other hand, race 24 which was first designated with four isolates in 1963 was identified as many as 36 times in 1964. It has become the most predominant race since then.

Most races designated in and after 1964 belonged to race groups of K, N, I and T. Only a few isolates were designated as races in P race group. In I race group. only four out of 53 isolates characterized during 1964-66 fell into the existing race patterns designated before 1962, i. e., races 9 and 11. The rest of isolates fell into the race pattern of newly designated, i. e., races 20, 21, 22 and 23 which were encountered with steady frequencies since 1963. No isolates were designated as races in J race group since 1964.

The patterns of race distribution in six localities where the uniform blast nurseries were located were quite similar. Among 27 races so far characterized, 18 races were encountered over all the localities, except Hualein area, during the years of 1960-66, The rest of the races were encountered in some localities with minor prevalence. Apparently, there was no local peculiarity in race distribution within the island of Taiwan.

The relative prevalence of a given race could be expected to change with shifts in acreage of susceptible rice varieties (3). In the uniform blast nurseries, the varieties of rice planted were changed from year to year. Susceptible varieties were screened out and not planted in the following years. More resistant rice varieties were provided for commercial use, especially blast resistant varieties of Ponlai rice which were distributed for increasing acreage of planting this type of rice (22). Most of Ponlai rice varieties inherited the blast resistant quality of native rice. It is assumed that increasing acreage of planting Ponli rice varieties brings on the shifts of races of the blast fungus in Taiwan.

It was evident that no isolates collected in and after 1964 showed infectious ability to the varieties of Japanese origin, namely Kanto 51, Noring 21 and Sensho in Taiwan differential varieties, except a few isolates in race 12. However, Kanto 51 received from IRRI as one of the eight international differentials was susceptible to some newly collected isolates in 1966, which were designated as the races other than race 12. This phenomenon might be due to the different origins of seeds of Kanto 51 in the international differentials and those in Taiwan differentials. The susceptiblity of this rice variety to the blast fungus might change during the successive cultivation in the different localities. In order to compare research information on an international basis, the seeds for differential varieties should be distributed to the investigators by a certain organization every year.

Evaluations of the U.S. and Japanese differentials were made with several representative

isolates in Taiwan races. The U.S. differential varieties did not provide a satisfactory differentiation for the Taiwan races. They differentiated some race pairs that show a general similarity in host reaction. However, they failed to distinguish some other races; several Taiwan races showed identical pathogenic pattern on the U.S. differentials. The Japanese differential varieties provided unequivocal separation of Taiwan races. Fourteen isolates representing 14 Taiwan races were distinguished from one another on Japanese differentials.

The Taiwan differentials provided a satisfactory differentiation for Japanese races. The reactions obtained with certain Japanese isolates on Taiwan differentials agreed with those recorded in tests made in Tokyo (27). It was evident that isolates of the same race charact erized by Japanese workers could be further differentiated by the Taiwan differential varieties. Among the 16 Taiwan differentials, *indica* type varieties seemed more valuable as differentials for U.S. races, while *japonica* type rice varieties mostly showed resistant reactions.

According to Takasaka *et al.* (28), the major races in Taiwan may consist of Japanese N-group races and of the Philippines races. He indicated that Taiwan race 2 and 17 were identical to Japanese N-4 on Japanese differentials. Having tested the *P. oryzae* isolates from Taiwan on Japanese and U. S. differentials, Goto and Kozaka reported the presence of Japanese races N-3, N-4 and N-5, and U.S. races 4 and 18 in Taiwan (2). The evidences were also indicated by Latterell *et al.* (18). They reported that U.S. races 18 and 21 isolated from specimens received from Taiwan appeared to have the same affinities as Japanese N-5 and N-1. In tests conducted in Taiwan, Taiwan races 12, 7 and 22 gave pathogenic pattern identical to Japanese N-6, N-2 and N-1, respectively on Japanese differentials, and Japanese race C-1, N-1, N-4 and N-5 showed the pathogenic patterns identical to Taiwan races 23, 22, 24 and 26 respectively on Taiwan differentials. Taiwan races 5, 12, 14, 13 or 16 and 17 showed pathogenic pattern identical or similar to the U.S. races 6, 21, 4, 17 and 2, respectively on the U.S. differentials, and the U.S. races 3 and 4 showed the reaction similar to race pattern of Taiwan race 2 on Taiwan differentials. Among U.S. races, races 2, 3, 4 and 21 are equavalent to Japanese N race group in the international differentials (4). Further evidences were obtained when the Taiwan races were tested on the international differentials. Most Taiwan races could be designated as races in IG and IH groups in which all prior Japanese N races were assigned. It is not surprising that the main races in Japan are present in Taiwan, since *japonica* type rice varieties are widely cultivated here. These facts might suggest that Taiwan and Japan share the same main races, prior N-race group. However, the blast disease in Taiwan has seldom caused serious damage as compared with that happened in Japan. The phenomenon that rice in 1st crop is more susceptible to the blast disease than in 2nd crop, might be due to the environmental factors influencing the growth of rice, which in turn show the different susceptiblity to the blast fungus. Lower temperatures during the 1st crop, which prolong the growth period of rice make it susceptible to the blast fungus. The susceptible period of rice in Taiwan is shorter than in Japan, and rice varieties, especially most of Ponlai rice of present cultivation inherited

the blast resistant quality from the native rice. Therefore less damage by the rice blast disease in Taiwan could be expected.

On the whole, more complex race patterns were obtained with Taiwan differentials than with the U.S. and Japanese differentials. The great complexity of rice varietal composition in Taiwan and the number of differentials employed for race characterization are the factors responsible for the complex race patterns (7). In individual countries where the eight international differentials are not sufficient for established rice-breeding program, the use of local subsidiary varieties was suggested by the U.S.-Japanese cooperatiue study (4). Since the present 16 Taiwan differentials have given a complex race pattern, and since most of the isolates collected in Taiwan were characterized into IG and IH groups, it is of necessity to select a set of superior differentials as a subsidiary varieties in Taiwan. Among the 16 Taiwan differentials, varieties which showed definite resistant or susceptible reaction to most of the existing blast races could be selected as candidate for subsidiary differential varieties. Varieties of Kanto 51, Pai-kan-tao, Chianung 242, Kao-chio-liu-chou, Taichung 65, and Kung-shan-wu-shen-ken in Taiwan differentials seemed to show a good differentiating ability. If these varieties are utilized as a set of differentials, isolates so far collected in Taiwan could be characterized into 17 races instead of the present 27 races.

As previously stated, most isolates of Taiwan races and newly collected isolates in 1966 were assigned as IG and IH race groups when the international differential varieties were used. The rest of the isolates with a minor instances were assigned as IC, ID, IE and IF race groups. No isolates tested were assigned as races in IA or IB groups. Atkins *et al.* (4) indicated that IA group of races occurs in Asiatic countries including Taiwan. However, according to the results of our tests, no isolates have been designated as races in the IA group. Since isolates which showed the IA pattern was obtained by the U.S. workers from specimen provided by the Taiwanese workers, the occurrence of this race in Taiwan is probable.

Summary

1) Nineteen races of *Piricularia oryzae* Cav., were characterized from 293 isolates of the blast fungus obtained during 1964-1966. Among them, 17 races representing 259 isolates showed the pathogenic patterns which had already been detected. The two newly chara cterized races (races 26 and 27) showed T race group patterns. Race 24 was the most prevalent race in 1964, being encountered 64 times. Another prevalent races of the period were races 17, 2, 3, 26, and 21.

2) Nine isolates representing six U.S. races and eleven isolates representing eight Japanese races were tested for their pathogenicity on the Taiwan differentials. Among the 16 Taiwan differentials, *indica* type varieties seemed more valuable as the differentials for the U.S. races than the *japonica* type varieties. Isolates of the same Japanese races did not give identical reactions on the Taiwan differentials. The pathogenic patterns on the Taiwan differentials, induced by Japanese race C-1 (Ken 60-19), N-1 (Hoku 373), N-4 (Ina-168) and N-5 (Naga 61-14) were identical to those by Taiwan race 23,

22, 24 and 26, respectively.

3) Fourteen isolates representing 14 Taiwan races were tested on U.S. and Japanese differentials. It seemed that the U.S. differentials did not provide a satisfactory differentiation for the Taiwan races. They failed to distinguish between race 13 and 16, race 9 and 22 or 24, race 14 and 17 or 19. On U.S. differentials, Taiwan race 5, 12, 14, 13 or 16, and 17 showed identical or similar pattern of U.S. race 6, 21, 4, 17 and 2, respectively. The 14 Taiwan races were unequivocally separated from one another on the Japanese differentials. On Japanese differentials, Taiwan race 12, 17, and 22 showed identical pattern of Japanese N-6, N-2, and N-1, respectively.

4) Some representative Taiwan races were tested on the 12 and 24 tentative international differentials supplied by USDA and IRRI respectively. In general, Taiwan races showed rather weak pathogenicity to these differential varieties. These sets of differen tials seemed not to provide a satisfactory differentiation for the Taiwan races.

5) Nine isolates representing nine Taiwan races of *P. oryzae* were tested on the 40 rice varieties supplied by FAO. All test isolates induced resistant reaction on varieties of group 1. Most of the varieties in groups 2 and 3 showed R reactions to the test isolates. Varieties in groups 4 and 5 showed inconsistent reactions. S reaction was caused by all the isolates in varieties of group 6.

6) Twenty commercial rice varieties were tested for their reactions to eight Taiwan races of *P. oryzae*. Among these varieties, only Taichung 186 and Taichung-yu 184 are resistant to all the races tested. The rest of the varieties are susceptible to most of the test isolates.

7) Several races of *P. oryzae* were obtained from one paddy field block consisting of one rice variety-Taichung 65. Races belonging to T race group were found to be the most prevalent ones.

8) The eight international differential varieties were tested for their reactions to the isola tes of *P. oryzae* which had been characterized with U.S., Japanese or Taiwan set of differentials, and to new isolates of the pathogen from Taiwan. Most of the former Taiwan races and newly collected isolates were included in IG and IH groups. Prior and new nomenclature designations for the former US and Japanese races are: US 1A=IB-5; US 5A, and US 6B=ID-8; US 7A=IF-1; US 3=IG-1; US 5B=IG-2; US 2 and 4=IH-1; T-1=IC-5; T-2=ID-10; C-1=IE-1; C-3=IF-2; N-1=IG-1; N-2, N-4, and N-5=IH-1.

Literature Cited

1. Atkins, J. G. 1962. Prevalence and distribution of pathogenic races of *Piricularia oryzae* in the U.S. Phytopathology 52 (1): 2 (Abstr.)
2. Atkins, J. G. *et al.* 1964. Report of conference on the U. S.-Japanece cooperative blast project, Plant Industry Station, Beltsville, Maryland, U.S.A. 10 p.
3. Atkins, J. G. 1965. Physiologic races of *Piricularia oryzae* in the Western Hemisphere, p.243-244. In The Rice Blast Disease. Proc. Symp. at the Int. Rice Research Inst., July 1963. Johns Hopkins Press, Baltimore.

4. Atkins, J. G. *et al.* 1967. An international set of rice varieties for differentiating races of *Piricularia oryzae.* Phytopathology 57: 297–301.
5. Chien, C. C. *et al.* 1964. Study on the physiologic races of *Piricularia oryzae* Cav. Final report, J.C.R.R. project, Taiwan Agr. Res. Inst. 16 p. (Mimeographed)
6. Chien, C. C., S. Y. Lin and S. C. Jong. 1963. Studies on the physiologic races of *Piricularia oryzae* Cav. (II) Agricultural Research (Taiwan). 12(3): 29–39.
7. Chiu, R. J., C. C. Chien and S. Y. Lin. 1965. Physiologic races of *Piricularia oryzae* in Taiwan. p. 245–255. In The Rice Blast Disease. Proc. Symp. at the Int. Rice Research Inst. July 1963, Johns Hopkins, Press, Baltimore.
8. Goto, K. 1965. Physiologic races of *Piricularia oryzae* in Japan, p. 237–242. In The Rice Blast Disease. Proc. Symp. at the Int. Rice Research Inst. July 1963. Johns Hopkins Press, Baltimore.
9. Goto, K. and T. Yamamura. 1961. Co-operative research on physiological races of *Piricularia oryzae.* Spec. Rep. No. 5. Studies on Insect and Disease Damage Spec. Suppl. 89. p.
10. Goto *et al.* 1964. Joint work on the race of rice blast fungas, *Piricularia oryzae* (Fascicle 2). Spec. report No. 18. Studies on Insect and Disease Damage Spec. Suppl. 132 p.
11. Hashioka, Y. 1952. Annual and local variation of the varietal resistance of rice to the blast disease; A criticism of the stability of varietal resistance based upon the field experiments in Taiwan. Agricultural Research (Taiwan) 3(3): 40–55.
12. Hung, C. H., C. C. Chien, and S. Y. Lin. 1961. Study on the physiological races of *Piricularia oryzae* Cav. Agricultural Research (Taiwan) 10(1): 27–34.
13. Ichikawa, M., M. Shimoyama, and M. Terazawa. 1957. Reaction of rice varieties in reference to inocula and method of inoculation. Ann. Phytopathol. Soc., Japan, 22:3.
14. Iwata, T., T. Narita, and S. Yamanuki. 1956. Research on races of rice blast fungus. Ann. Phytopathol. Soc., Japan, 21(1): 36–37. (Abstr.)
15. Kou, T. T., S. C. Woo, and W. H. Wang. 1963. Some physiologic specializations of *Piricularia oryzae* Cav. in Taiwan. Acad., Sinica Bot. Bull. 4: 23–29.
16. Hemmi T. 1949. Studies on rice blast disease. Asakura Co., Tokyo, p. 269–93.
17. Kuribayashi, K., *et al.* 1959. Studies on the races of rice blast fungus, *Piricularia oryzae* Cav., with reference to differential varieties of rice plants and spray or injection for inoculation. Nagano Pref. Agr. Expt. Sta. Proc. 2: 127–42.
18. Latterell, F. M., *et al.* 1965. Co-ordination of effort to establish an international system for race identification in *Piricularia oryzae.* p. 257–274. In The Rice Blast Disease. Proc. Symp. at the Int. Rice Research Inst., July 1963, Johns Hopkins, Press, Baltirmore.
19. Latterell, F. M., E.C. Tullis, and J. W. Collier. 1960. Physiologic races of *Piricularia oryzae* Cav.:Plant Dis. Reptr. 44(9): 679–683.
20. Latterell, F. M., E. C. Tullis, R. T. Otten, and A. Gubernik. 1954. Physiologic races of *Piricularia oryzae.* Phytopathology 44: 495 (Abstr.)

21. Li, H. C. 1957. Progress report on the study of physiologic races of *Piricularia oryzae* Cav. in Taiwan. Taiwan Agr. Res. Inst. (Mimeographed).
22. Lin, S.C., and C. W. Yang. 1967. The Change of rice varieties and cultivative tendencies of Ponlai varieties in Taiwan. Taiwan Agricultural Quarterly 3(1): 44-50.
23. Nakanishi, I. 1957. Studies on the race of rice blast fungus; method of inoculation. Ann. Phytopathol. Soc. Japan, 22(1): 3
24. Sasaki, R. 1922, 1923. Existence of strains in rice blast fungus. I & II. J. Plant Protect. 9: 631-44, 10: 1-10.
25. Shimoyama, M. 1960. Change of pathogenicity of *Piricularia oryzae* through successive cultures. Proc. Assoc. Plant Protect. Hokuriku, 8: 79-82.
26. Suzuki H. 1967. Studies on biologic specialization in *Piricularia oryzae* Cav. Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tokyo.
27. Takasaka, T. 1964. Personal communication.
28. Takasaka, T. *et al.* 1964. Reactions of Japanese differential varieties to Taiwan races of *P. oryzae*. Ann. Phytopathol. Soc. Japan, 29(2): 55.
29. Tochinai, Y. and T. Nakano. 1940. Studies on the nutritional physiology of *Piricularia oryzae* Cav. J. Fac. Agr. Hokkaido Univ. 44: 183-299.
30. Tochinai, Y. and M. Shimamura. 1932. Studies on the physiological specialization in *Piricularia oryzae* Br. *et.* Cav., Ann. Phytopathol. Soc., Japan, 2(5): 414-41.
31. Yamanaka, S. 1963. Studies on the race of rice blast fungus. Sorting out of isolates strongly pathogenic to the highly resistant rice strain, Pi No. 1.; Ann. Phytopathol. Soc., Japan, 28(2): 61. (Abstr.)

PART II

THE VITAMIN, CARBON AND NITROGEN NUTRITION OF *PIRICULARIA ORYZAE* CAV.

S. Y. Lin, and C. C. Chien[1]

Introduction

The existence of physiologic races of the rice blast fungus has been reported from different rice growing countries. (3, 4, 10, 11, 13, 16, 17, 18). In Taiwan, numerous isolates of *P. oryzae* have been characterized into physiologic races on the basis of their pathogenicity to the differential rice varieties (7, 8, 12, 15).

Isolates of *P. oryzae* also differed in their physiologic characteristics such as mycelial growth, pigmentation, conidium production, and nutritional requirement. Several workers (1, 7, 12, 28) compared the cultural characteristics of a number of the rice blast fungal isolates and classified them into different cultural types. Suzuki (24) analyzed a large number of single spore isolates and grouped them into 11 biologic species on the basis of appressorium type. Chien *et al* (7) found that cultural types of the blast fungus were to some extent associated with pathogenicity, but no definite correlation was observed between cultural characters and pathogenic races.

Many workers have studied the effects of carbohydrates (2, 5, 6, 14, 22, 23, 25, 29, 30), nitrogen sources (2, 5, 6, 14, 20, 21, 23, 25, 27, 29) and vitamins (2, 14, 21, 23) on the growth of *P. oryzae*. Among them, Otsuka in Japan, suggested the close relationship between relative strength of pathogenicity and nutrition of the fungus. He assumed that the difference in pathogenicity shown by races of *P. oryzae* was caused by differences in their metabolical mechanism and biochemical properties, and that the differences in utilization of nutrition were considered to be the result of biochemical differences among the strains, and were available as a basis of classification (23). Chen (6) also stated that the sensitivity of *P. oryzae* to the nutritional status of the host was partly due to its specific nutritional requirements. Others workers (14, 30) reported that nutrition of *P. oryzae* varied in different isolates and this variation was similar to the variation in virulence of the isolates to different rice varieties

The primary purpose of the investigations was to study the vitamin, carbon and nitrogen nutrition of *P. oryzae* and to elucidate the possible correlation between nutrition and pathogenicity of the fungus.

---

1. Associate Plant Pathologists, Department of Plant Pathology, Taiwan Agricultural Research Institute, Taipei, Taiwan, China.

Meterials and Methods

Isolates of *P. oryzae* showing distinct degree of pathogenicity were used. They included ten Taiwan isolates, one Japanese and one U.S. isolate.

The basal medium consisted of KNO_3 2 g, KH_2PO_4 0.5 g, K_2HPO_4 0.5g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $CaCl_2 \cdot 6H_2O$ 0.1 g, $FeSO_4 \cdot 7H_2O$ 0.0075 g, $CuSO_4 \cdot 5H_2O$ 0.007 g, $MnSO_4 \cdot 6H_2O$ 0.002 g, $ZnSO_4 \cdot 7H_2O$ 0.009 g, H_3BO_3 0.0009 g, dextrose 30 g, and distilled water to make up one liter. When the effect of carbon or nitrogen compound on the growth of the fungus was studied, 2 mg. thiamine and 5 μg biotin were added to one liter of the basal medium.

Vitamins were added to give the final concentration indicated: thiamine 2 mg, biotin 0.005 mg, riboflavin 0.5 mg, pyridoxine hydrochloride 0.5 mg, calcium pantothenate 2 mg, p-aminobenzoic acid 0.5 mg, inositol 4 mg, nicotinic acid 5.0 mg, folic acid 0.004 mg, choline chloride 2 mg, and ascorbic acid 0.5 mg in one liter medium. To determine optimum concentration of thiamine and biotin for growth, concentrations listed in Table 4, and 5 were utilized.

The carbon or nitroge sourses were prepared to yield the amount of carbon equivalent to that in 30 g of glucose, and the nitrogen compound equivalent to that in 2 g potassium nitrate per liter of medium.

All media were sterilized by Seitze filtration, unless otherwise stated. In preliminary experiments, the solid media were sterilized at 100°C for 30 minutes thrice at 24 hours interval. The pH of media was measured by Leeds pH meter and adjusted to pH 7.0 by adding 1.0 N NaOH. The pH values of the residual liquid media was determined at the end of all experiments.

The fungus was grown in 30 ml of liquid medium contained in 125 ml Erlenmeyer flask. Some experiments were conducted in solid media, which were prepared by adding 2% Difco Bacto-agar and distributed in square flasks.

Inoculum used was a mycelial disc cut from the edge of a 14-day-old colony on the vitamine-free basal medium with a 5 mm cork borer. Stationary cultures were utilized in all experiments. Samples on liquid media were harvested after 20 days incubation at 28°C. The compact mycelial mat was washed several times with distilled water. A water suction pump was used to speed the harvest. The mycelial mat from the quintuplicates were collected respectively. After drying the mycelial mat at 60°C for 24 hours on previously dried and weighed filter paper, the dry weight of mycelium was determined. The results are presented as the average weights of the five replicates in mg dry weight.

To determine spore production on solid media, ten 5 mm diameter mycelial discs were cut from each colony after 7 and 14 days incubation at 28°C, and transferred into a Kahn tube containing one ml distilled water. The mycelial discs in the tube were homogenized and shaken vigorously and spore count was made with a hemocytometer.

Results

Effect of vitamins: In initial experiments to determine which among the 11 vitamins was responsible for the growth and sporulation of the fungus, the media were prepared so

that each of the 11 vitamins was withheld in turn, the other 10 vitamins being present. There are two controls, one the basal medium only, and the other the basal medium containing all 11 vitamins. The results are given in Table 1. Among the 11 vitamins used only biotin and thiamine were influential to the growth of the fungus. Other vitamins were found ineffective. The dependency of the fungus on biotin was greater than on thiamine. In the biotin free medium, most of the isolates showed very slight mycelial growth. Some isolates such as U.S. 5A, 4S-26S, and 4T-28S required much more exogenous biotin than other isolates. They gave almost no growth on biotin-free medium. The dependency of the fungus on thiamine varied from almost a complete requirement to partial requirement depending on the isolates. It was found that a majority of the isolates had a partial requirement for thiamine. Some isolates appeared to have almost no thiamine requirement, whereas most of the other isolates showed varying degrees of deficiency for thiamine. However, no isolate exhibited an absolute requirement for this vitamin. In the thiamine-free medium, isolates 4S-26S and 4C-10S produced as much mycelium as that in "all" medium. Three isolates i.e., 4S-15S, 3C-58G,and 4K-4S produced 1/3-1/2 of mycelial dry weight, and the other four isolates, 0'S-45S, 4T-28S, Ken 60-19 (C-1), and U.S. 5A gave 2/3 growth as compared with that in "all" medium.

In general, isolates of *P. oryzae* in the media devoid of vitamins other than biotin or thiamine produced the dry weight of mycelium as much as in "all" medium. Some isolates, such as isolate 3C-58G for example, produced more dry weight of mycelium in media devoid of ascorbic acid or p-aminobenzoic acid. From these results, it was apparent that none of the fungal isolates showed a multiple vitamin requirement with regard to the 11 vitamins used in this study. Only biotin and thiamine, especially the former, were found to be indispensable to the growth of the fungus.

Sporulation of the fungus varied greatly with isolates and with media. Generally, spores were produced much more abundantly after 14 days incubation. In some instances more spores were produced in the medium devoid of a certain vitamin than in "all" medium. Biotin and thiamine also greatly influenced the sporulation of the fungal isolates. No conidium was produced by any isolates in either biotin or thiamine-free media. It was demonstrated that both biotin and thiamine were absolutely essential for the sporulation of *P. oryzae*. (Table 2)

Table 1. Growth of *P. oryzae* in a basal medium supplemented with different vitamins and glutamic acid.

Vitamin	Isolate									Treatment mean
	(1) 3C-58G	(12) 4S-15S	(13) O'S-45S	(16) 4S-26S	(20) 4C-10S	(22) 4T-28S	(24) 4K-4S	Ken 60-19 (C-1)	U. S. 5A	
All[a]	137[c]	205	144	158	154	150	171	130	122	152.3
All−thiamine	61	68	100	142	118	92	88	89	85	93.7
All−biotin	32	35	35	15	38	19	30	34	3	26.8
All−inositol	173	197	145	178	156	135	180	132	141	159.7
All−riboflavin	163	158	139	175	129	164	175	154	140	155.2
All−calcium pantothenate	179	186	157	156	161	146	183	156	147	163.4
All−ascorbic acid	183	164	142	142	156	157	155	145	131	152.8
All−nicotinic acid	150	200	144	153	181	143	175	143	124	157
All−folic acid	173	206	125	129	147	154	175	162	117	154.2
All−p−aminebenzoic acid	183	219	129	150	184	125	177	143	120	158.9
All−pyridoxine hydrochloride	156	194	134	145	114	141	171	113	126	143.8
All−choline chloride	164	174	137	98	165	144	161	126	138	145.2
Basal media only	13	36	5	2	27	8	41	21	5	17.6
B.M.[b]+thiamine+biotin	139	296	131	171	157	160	181	137	138	167.8
B.M.+thiamine+biotin+glutamic acid	170	200	131	103	181	152	200	160	131	158.7
Isolste mean	138.4	169.2	119.9	127.8	137.9	126	150.9	123	111.2	

a Basal medium plus 11 vitamins.
b The basal medium.
c Dry weight of mycelium in mg.
L.S.D. 0.05 between treatments=19.2
0.01 between treatments=25.5
L.S.D. 0.05 between isolates=21.8
0.01 between isolates=30.4

Table 2. Sporulation of *P. oryzae* in a basal medium supplemented with different vitamins and glutamic acid.

Vitamin	Isolate																Treatment mean	
	(1) 3C-58G		(12) 4S-15S		(16) 4S-26S		(20) 4C-10S		(22) 4T-28S		(24) 4K-4S		(26) 5K-33S		U.S. 5A			
All[a]	0.8[b]	2.4[c]	2.3	3.5	31.2	78.7	2.2	4.3	26	12.8	0.8	14.7	1.9	9.3	26.1	113.5	11.41	29.9
All−thiamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
All−biotin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
All−inositol	0.9	1.5	5.1	7.0	27.6	33.8	2.8	9.1	51.3	30	1.6	21.6	3	3.4	13.6	132	13.24	31.05
All−riboflavin	2.2	1.2	6.2	8.2	50.7	30.6	5.5	2.2	28.5	18.8	2	16.1	0.8	5.3	35.1	139.1	16.38	27.69
All−calcium pantothenate	1.2	2.1	4.3	4.5	30.0	27.3	2.2	1.5	37.6	15.6	2.8	11.8	1.9	4.3	16	105.4	12	21.56
All−ascorbic acid	0.5	1.6	9.5	8.3	16.4	114.3	2.1	3.8	24.8	23.9	1.5	32	0.7	11.9	35.5	140.9	11.38	32.59
All−nicotinic acid	1.2	2.5	5.6	6.4	43.2	24.1	4.9	3.3	21.8	20.1	0.5	19.8	0.5	4.6	19.7	151.7	12.18	29.06
All−folic acid	2.3	1.5	4.5	6.4	49.8	17.1	1.3	1	28.5	35.8	3.6	24.1	2.5	9.4	82.7	138.7	21.9	39.25
All−p−aminobenzoic acid	0.9	2.3	5.2	5.5	45.7	88.3	2.5	5.2	35.5	32.4	1.7	15.7	1.6	2.8	23.8	109.5	14.61	32.71
All−pyridoxine	0.8	2.4	7.2	8.2	27.8	43.6	5.3	8.8	33.3	19.5	1.9	20.7	3.0	5.6	15.3	114.4	11.83	27.9
All−choline chloride	1.4	1.5	5.5	6.0	29.7	25.8	3	4.9	37.1	34	5.7	16.3	2.3	3.2	6	76	11.34	20.96
B.M. only	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B.M.+thiamine+biotin	0.9	2.2	4.8	5.0	14.9	44.2	4.8	7.4	27	32	2.4	15.5	0.8	8.5	4.9	11.8	7.56	15.83
B.M.+thiamine+biotin+glutamic acid	0.6	1.1	9.5	12.2	15.7	6.3	5.5	16.3	13.6	49.2	0.6	7.3	0.4	17.5	17.3	121.9	9.03	28.98
Isolate mean	0.9	1.5	4.7	5.4	25.5	35.6	2.8	4.5	24.3	21.6	1.7	14.4	1.9	6.4	19.7	90.3		

a Basal medium plus 11 vitamins.
b Number presented in 20 thousands of conidia produced on ten 5mm diameter agar discs after one week incubation.
c Number of conidia produced after two weeks incubation.
One week incubation:
L.S.D 0.05 between treatments=35.18
0.01 between treatments=46.79
no significant differences between isolates
Two weeks incubation:
L.S.D 0.05 between treatments=11.26
0.01 between treatments=34.84
L.S.D. 0.05 between isolates=13.21
0.01 between isolates=43.47

In subsequent experiments, vitamin was individually added to the basal medium which contained thiamine and biotin. All isolates gave 1/2—2/3 mycelial growth in the medium with biotin alone, as compared with those in the medium containing both thiamine and biotin. In the medium devoid of biotin, isolates 4T-28S and U.S. 5A showed almost no growth, the rest of the isolates, gave only slight growth. Addition of any other vitamin in the medium containing biotin and thiamine did not further increase the fungal growth. This fact clearly indicated that no appreciable growth was secured with a single vitamin other than biotin and thiamin. When biotin was used in association with thiamine, the fungal growth was greatly increased. (Table 3).

It may be concluded that biotin was indispensable to the growth of the fungus. Thiamine was only a complimentary growth factor. In the absence of thiamine, several isolates of the fungus were able to make fairly abundant mycelial growth but failed to produce spores. Thus, thiamine was more important for sporulation than for vegetable growth. Thiamine alone could exert certain degree of mycelial growth in some isolates, yet thiamine effected fungal growth and especially sporulation only when a sufficient amount of biotin was present in the medium.

Leaver *et al* (19) reported that both thiamine and biotin were essential for growth of the fungus and that the optimum concentration of biotin was 1.0-10 μg/1. Otani (21) stated that biotin was essential for the growth of *P. oryzae*, its optimum concentration being 2.3-3.0 μg/1. In our studies, the minimum concentration of biotin for the optimum growth of *P. oryzae* was 0.5 μg/1, and of thiamine was 0.02 mg/1. Below these concentrations, the growth of the fungus was drastically reduced. (Table 4, Table 5). It was noted that different isolates of the fungus required different amounts of the same vitamin for optimum growth, however, no significant difference in the growth of a given isolate was observed

Table 3. Growth of *P. oryzae* in a basal medium supplemented with biotin, thiamine and other vitamins

Vitamin	Isolate						Vitamin mean
	(1) 3C-58G	(13) 0'S-45S	(22) 4T-28S	(24) 4K-4S	(26) 5K-33S	U.S. 5A	
B.M.[a]	15[c]	10	5	14	20	8	12
B.M.+biotin	65	102	100	88	77	94	87.7
B.M.+thiamine	28	24	8	27	16	5	18
B.M.+B.T.[b]	188	159	197	187	182	177	181.7
a+b+inositol	171	157	180	191	200	185	180.7
a+b+riboflavin	131	158	194	177	180	188	171.3
a+b+calcium pantothenate	196	142	171	178	176	171	172.3
a+b+ascorbic acid	160	159	185	205	215	156	180
a+b+nicotinic acid	156	167	201	197	188	166	179.2
a+b+folic acid	165	165	184	226	186	153	179.8

a+b+P-aminobenzoic acid	170	188	186	164	190	187	180.8
a+b+pyridoxine hydrochloride	187	145	176	166	179	182	172.5
a+b+cholinc chloride	187	195	186	150	188	150	176
Isolate mean	139.9	136.2	151.8	151.5	153.6	140.2	

a. The basal medium
b. Biotin and thiamine
c. Dry weight of mycelium in mg.
L.S.D. 0.05 between treatments=23.70
0.01 between treatments=31.52
L.S.D. 0.05 between isolates =29.38
0.01 between isolates =42.11

Table 4. The influence of biotin concentration on the growth of *P. oryzae*.

concentration (μg/l.)	(1) 3C-58G	(13) 0'S-43S	(22) 4T-28S	U.S. 5A	Biotin concentration mean
5,000	218[a]	220	210	142	197.5
500	264	220	215	138	209.3
50	200	216	214	128	189.5
5	239	215	195	126	193.8
0.5	125	160	207	144	159.0
0.05	45	52	42	30	42.3
0.005	45	47	25	39	39.0
0.0005	48	45	20	30	35.8
Isolate mean	148	146.9	141	97.3	

a. Dry weight of mycelium in mg.
L.S.D. 0.05 between treatments=39.67
0.01 between treatments=53.97
L.S.D. 0.05 between isolates =48.91
0.01 between isolates =72.01

Table 5. The influence of thiamine concentration on the growth of *P. oryzae*.

concentration (mg/l.)	(1) 3C-58G	(13) 2C-2124S	(22) 4T-28S	U.S. 5A	Thiamine concentration mean
20	220[a]	285	195	186	221.5
2.0	213	310	190	213	231.5
0.2	143	256	185	147	182.8
0.02	95	200	95	105	123.8
0.002	84	120	95	72	92.8
0.0002	56	65	92	62	68.8
Isolate mean	135.2	206	142	130.8	

a. Dry weight of mycelium in mg.
L.S.D. 0.01 between treatments=39.49
0.01 between treatments=54.69
L.S.D 0.05 between isolates =44.80
0.01 between isolates =65.00

with either biotin or thiamine in concentrations above minimum requirement. The minimum requirement of biotin for isolates 3C-58G and 0'S-45S was 5 μg/1, and for isolates 4T-28S and U.S. 5A, 0.5 μg/1. (Table 4). The minimum requirement of thiamine for isolate 3C-58G was 2 mg/1, for isolates 4T-28S and U.S. 5A, 0.2 mg/1, and for isolate 2C-2124S, 0.02 mg/1 (Table 5.).

Effect of carbon sources: The results of carbon nutrition of *P. oryzae* are given in Table 6. The ability of most of the isolates to utilize certain carbon compounds were relatively similar in many instances. Most of the isolates well utilized glucose, sucrose, fructose, starch, maltose, and lactose, and the variation in utilization of these compounds among the isolates was not great. The ability to utilize galactose, dextrin, and glycerol, however, differed with isolates. Galactose was a poor carbon source for most of the isolates, yet its utilization by isolate 3C-58G and 4C-26S was satisfactory. Sorbose was also unsuitable for the fungus growth. Raffinose supported only moderate growth of the fungus. Among polysaccharides tested, only starch was a suitable carbon source for all isolates, while inulin and cellulose were not Dextrin was utilized moderately by all isolates. Alcohols and organic acids were the poor sources of carbon. It was of interest to note that isolate 6C-34S utilized cellulose and glycerol far better than the rest of the isolates. These two compounds were poorly utilized by the rest of the isolates. Rather fluctuating results were shown when xylose, lactose and starch were used as carbon sources. There were no apparent differences in the utilization of single carbon source by the ten isolates. Differences in the growth of a single isolate were recorded, however, when it was grown on different carbon sources.

Effects of nitrogen sources: In the present studies, various nitrogen compounds were utilized to varying extent by 10 isolates of the fungus (Table 7). Among the inorganic nitrogen compounds, nitrate was utilized very well by all isolates, even nitrite was shown as a good nitrogen scurce for the fungus growth. On the other hand, poor growth of the fungal isolates occurred in the ammonium form of nitrogen sources. Suitability of nitrate-N over ammonium-N was reported by Tanaka *et al.* (27), who stated that the inferiority of ammonium-N was caused by the fall of pH in the medium. In our studies, it was the only exception that isolate 5K- 33S produced large amount of mycelium when NH_4Cl was used as the nitrogen source. Urea was also a suitable nitrogen source. All isolates, except 5K-33S, showed good growth in the medium containing urea. Among amino acid tested, glutamine, glutamic acid, asparagine, aspartic acid, leucine, glycine, and serine were well utilized by all isolates. Most isolates utilized alanine and proline to some extent, while poor utilization was shown by 2K-27S in alanine and by 3C-58G in both amino acid media. Utilization of histidine, valine, arginine and methionine, varied with isolates. Tryptophane was utilized well by all isolates with the exception of 4T-28S. Cystein was the most unsuitable nitrogen source for the growth of all isolates. Peptone and casein hydrolysate were also found to be the suitable nitrogen sources. There were no apparent differences among the isolates when the utilization of nitrogen compounds were considered. There were, however, notable differences in yield of mycelium obtained with the different nitrogen compounds for a given isolate.

Table 6. Utilization of carbon sources by 10 isolates of *P. oryzae*

Carbon sources	Isolate										Carbon source mean
	(1) 3C-58G	(3) 2K-27S	(12) 4S-15S	(13) 0'S-45S	(16) 4S-26S	(22) 4T-28S	(24) 4K-4S	(26) 5K-33S	6C-34S	U.S. 5A	
Glucose	203[c]	175	210	215	167	149	184	169	189	200	186.1
Fructose	170	181	187	142	194	173	204	193	181	222	184.7
Galactose	160	29	43	59	120	22	55	37	35	36	59.6
Sorbose	50	30	29	42	47	62	30	36	24	34	33.4
Xylose	125	112	108	79	114	155	150	164	210	205	142.2
Sucrose	228	189	248	210	200	159	195	214	200	213	205.6
Maltose	160	172	151	125	227	137	172	190	194	148	167.6
Lactose	178	155	114	156	108	99	84	105	143	107	124.9
Raffinose	90	64	80	70	68	93	94	100	61	87	80.7
Starch	263	134	216	256	178	166	195	127	153	123	181.1
Dextrin	105	95	105	116	80	95	65	95	93	92	94.1
Inuline	30	28	41	47	53	42	33	38	28	52	40.1
Cellulose[a]	8	10	35	43	35	22	21	6	72	26	27.8
Mannitol	45	40	85	20	40	27	23	42	28	92	44.2
Glycerol	25	40	40	13	34	74	27	40	123	78	49.4
Na-Succinate	95	50	35	37	56	29	30	39	45	33	44.9
Na-Citrate	79	40	31	35	60	24	25	50	21	19	38.4
Control[b]	13	5	10	14	20	8	15	10	12	8	11.5
Isolate mean	112	86.7	99.3	95.3	101.4	85.3	90.2	91.9	102.3	98.6	

a. The medium was sterilized at 100° C for 30 minutes thrice at 24 hours interval.
b. Control medium contains no carbon compound.
c. Dry weight of mycelium in mg.
L.S.D. 0.05 between treatments = 24.92
0.01 between treatments = 32.99
L.S.D. 0.05 between isolates = 28.27
0.01 between isolates = 39.37

Table 7. Utilization of nitrogen sources by 10 isolates of *P. oryzae*

Nitrogen source	Isolate										Nitrogen source mean
	(1) 3C-58G	(3) 2K-27S	(12) 4S-15S	(13) 0'S-45S	(16) 4S-26S	(22) 4T-28S	(24) 4K-4S	(26) 5K-33S	6C-34S	U.S. 5A	
KNO_3	200[b]	210	212	234	183	157	110	110	195	212	183.2
$NaNO_3$	182	202	206	247	177	175	140	133	165	210	183.7
$NaNO_2$	195	202	187	133	236	160	152	207	169	192	183.3
NH_4Cl	65	50	70	47	45	30	64	148	29	30	57.8
NH_4NO_3	60	50	84	53	40	38	60	62	50	48	54.5
$(NH_4)_2SO_4$	50	45	96	65	78	70	60	52	51	81	64.8
Urea	110	120	174	141	140	151	117	58	178	190	137.9
Glutamic acid	226	240	234	215	194	166	124	116	155	225	189.5
Glutamine	171	190	165	242	187	276	168	161	173	236	196.9
Aspartic acid	181	197	240	280	212	280	149	196	187	278	220.0
Asparagine	203	222	201	270	180	174	114	205	200	254	202.3
Histidine	123	111	94	85	57	80	64	75	79	159	92.7
Valine	76	50	111	203	95	94	163	93	60	86	103.1
Alanine	75	89	236	218	112	188	147	201	235	176	167.7
Proline	85	285	181	158	158	222	188	268	173	236	195.4
Leucine	185	203	190	162	108	190	176	235	190	273	191.2
Glycine	240	219	160	156	213	200	173	240	123	232	195.6
Arginine	86	50	152	142	136	180	62	100	70	98	107.6
Methionine	60	52	180	151	80	158	80	186	100	102	114.9
Tryptophane	165	126	171	175	108	85	175	138	192	153	148.8
Serine	188	123	157	220	201	189	178	236	173	212	187.7
Cystein	65	55	77	86	40	23	21	32	30	25	45.4
Casein hydrolysate	176	134	182	196	160	140	154	158	163	120	158.3
Peptone	160	153	189	226	125	106	169	130	164	180	160.2
Control[a]	42	14	18	38	38	21	20	38	25	18	27.2
Isolate mean	138.3	139.3	158.7	165.7	132.1	142.1	121.1	143.1	133.2	161.0	

a. The control medium contains no nitrogen compound.

b. Dry weight of mycelium in mg.

L.S.D. 0.05 between treatments = 25.75

0.01 between treatments = 30.83

L.C.D. 0.05 between isolates = 31.49

0.01 between isolates = 40.10

Discussion

Studies on vitamin nutrition of *P. oryzae* showed that among the eleven vitamins tested only biotin and thiamine were influencial to the growth and sporulation of the fungus. The fungus seemed to have no requirement for the other vitamins. None of the fungal isolates showed a multiple vitamin requirement with regard to the 11 vitamins used in this study. It was found that dry weights of mycelia produced by the isolates in the biotin-free medium and in the basal medium were almost the same, while more mycelia was produced in the thiamin-free medium. The results that isolates of *P. oryzae* required biotin as an indispensable growth factor, and thiamine as a complimentary growth factor coincided with the results obtained by the other workers (21, 23, 26). Most isolates absolutely required an exogenous supply of biotin. Some isolates had only a negligible requirement for thiamine. Different degree of deficiency for biotin or thiamine may reflect different rates of synthesis of these vitamines in the fungal mycelium. Cochrane (9) stated that fungi not requiring or requiring only a negligible amount of exogenous supply of a vitamin must synthesize the compound and that partial deficiencies for a vitamin are probably more common than absolute or complete deficiencies. When fungi synthesize more of a vitamin that they require for optimum growth, the excess may accumulate in the mycelium or excrete into the culture medium (23). Some isolates produced significantly more mycelium in certain vitamin-free medium than in "all" medium. This might be due to the suppressing factor of the vitamin on the growth of the fungus. It needs further study to clarify whether or not these suppressing effects are present.

From the results of sporulation test, it was clearly shown that biotin and thiamine are absolutely essential for the sporulation of *P. oryzae*. In the absence of these two vitamins, several isolates were able to make some mycelial growth, but failed to produce spores. Apparao (2) also found that larger quantities of thiamine and biotin were required for sporulation than for growth. Sporulation of the fungus varied greatly with isolates and with media, yet, to what degree the vitamins other than thiamine and biotin influenced the sporulation of the fungus is not clear.

Otsuka (23) indicated that the requirement of biotin, thiamine and nicotinic acid of strains of *P. oryzae* was closely related to the relative strength of pathogenicity. According to him the majority of isolates which grew only in medium containing both biotin and thiamine were considered to have medium or strong pathogenicity. A few isolates which grew in media devoid of either biotin or thiamine had weak or no pathogenicity. Isolates with strong pathogenicity generally grew in the medium containing biotin and nicotinic acids. In the present study, because of limited number of test isolates, it seemed too early to draw the conclusion. Nevertheless, from our results, it could be inferred that no clear correlation was observed between the pathogenicity and vitamin requirement of the rice blast fungus.

Many workers have studied the carbon nutrition of *P. oryzae*. In general, organic acids

were not suitable carbon sources for the fungus (14, 22, 30). The same results were obtained in our studies. Otani (22) however, reported that glucose, inulin and mannitol, as well as organic acid such as succinic acid are the most excellent carbon sources for the fungus growth. Inulin and mannitol were found to be a poor carbon sources by the present writers. Glycerol was regarded as an unsuitable nutrient by Tomizawa (29), but was reported as an excellent carbon source by Tanaka (25). In our studies, it was noted that most isolates were unable to utilize glycerol, with the exception of isolate 6S-34S which utilized glycerol quite well. According to Otani (22), lactose was unfavorable carbon source. Other workers, however, reported that lactose was well utilized by the fungus (5, 6, 14, 23). Xylose was reported as a suitable carbon source by several workers (14, 22). It was also found to be a relatively poor source of carbon (30). In our studies, all isolates, except 0'S-45S, utilized xylose to a certain extent. Otsuka (23) indicated that there were marked differences among strains in utilization of sorbose, inulin and mannitol. According to him the strongly pathogenic group were inulin, cellulose utilization positive and sorbose utilization negative, and those strain which showed weak pathogenicity belonged to inulin, cellulose utilization negative, sorbose utilization positive group. Chen (5) also reported that isolates showed higher ability in cellulose utilization gave higher pathogenicity. In our studies, there were no apparent differences in the utilization of these carbon sources by the isolates, although utilization of cellulose somewhat differed with isolates.

The blast fungus utilized a wide variety of inorganic and organic nitrogen compounds. In the present study, nitrate and nitrite nitrogen was superior to ammonium nitrogen. $NaNO_2$ was found to be unfavorable for the growth of the fungus (5, 20, 21). Otsuka (23) reported that some strains of the blast fungus grew well in a medium containing $NaNO_2$, while others did not. He stated that strains that did not utilize $NaNO_2$ or utilize it poorly, mostly showed strong pathogenicity, while those strains that utilized it well showed weak pathogenicity. In the present studies, all isolates tested utilized $NaNO_2$ satisfactory, and the difference among isolates was not apparent. Many workers (14, 20, 21) reported that ammonium-N was unsuitable nitrogen source. Tanaka (27) ascertained that the inferiority of ammonium-N was caused by the fall of pH value in the medium. By contrast, Chen (6) stated that ammonium salts were more favorable inorganic N sources than nitrate. Among amino acid tested, glutamine, glutamic acid, asparagine, aspartic acid, leucine, glycine, and serine were well utilized by all isolates. These results are similar to those reported elsewhere (5, 14, 20, 23). Generally, the leaves of susceptible rice plant have higher content of amino acids (25). This may suggeste a possible relationship between the growth responses of *P. oryzae* in culture to amino acids and the establishment of the fungus in the host. It was pointed out by Otsuka (23) that strain of strong pathogenicity generally utilized trytophane poorly, while those of weak pathogenicity generally utilized it well. In our studies, however, no difference in tryptophane utilization was observed among isolates of different pathogenicity. Most isolates utilized tryptophane well, with the exception of isolate 4T-28S. No clear correlation was found between utilization of nitrogen or carbon compounds and the virulence of the isolates.

Summary

Vitamin requirement for growth and sporulation of a certain isolates of *Piricularia oryzae* Cav. were determined. Of the vitamins studied, only biotin and thiamine were essential to the growth of the fungus. Other vitamins were found ineffective. The dependency of the fungus on biotin was greater than on thiamine. All isolates tested required exogenous biotin for mycelial growth. It was found that a majority of the isolates had a partial requirement for thiamine. No isolates exhibited an absolute requirement for thiamine. Both biotin and thiamine were absolutely essential for the sporulation of *P. oryzae*. No conidia were produced by any isolate in either biotin or thiamine free media. In the absence of thiamine, several isolates of the fungus were able to make fairly abundant mycelial growth, but failed to produce spores. The minimum concentration of biotin for the optimum mycelial growth of the fungal isolates was 0.5 μg/1, and of thiamine, 0.02 mg/1. Below these concentrations, the growth of the fungus was drastically reduced. No correlation was observed between pathogenicity and vitamin requirement of the fungus.

The effects of different carbon and nitrogen compounds upon the growth of the fungus were also studied. The best carbon sources for all isolates were glucose, sucrose, fructose, starch, maltose, lactose and xylose. Raffinose was utilized moderately by all isolates. Galactose and Sorbose were found to be unfavorable for most of the test isolates. Alcohols and organic acids were the poor sources of carbon. With the exception of starch, all polysaccharides studied were unfavorable for the fungus growth. Utilization of cellulose by isolates 6C-34S, 0'S-45S and 4C-15S was somewhat better than the other isolates. Dextrin supported only moderate growth of the fungus. Among the inorgainc nitrogen tested, nitrate and nitrite nitrogen were superior to ammonium. Several amino acids such as glutamine, glutamic acid, asparagine, aspartic acid, leucine, glycine, and serine were well utilized by all isolates. Cystein was the most unfavorable amino acid tested. Tryptophane was utilized well by all isolates with the exception of 4T-28S. Most isolates utilized alanine and proline to some extent. Utilization of histidine, valine, arginine and methionine varied with isolates. There were no apparent differences among the isolates when the utilization of nitrogen and carbon sources were considered.

Literature Cited

1. Aoki, Y. 1935. On the physiological specialization in the rice blast fungus, *Piricularia oryzae* Cav. Ann. Phytopath. Soc. Japan 5: 107-120.
2. Apparao, A. 1962. Nutritional factors governing Sporulation of *Piricularia oryzae* in culture. J. Indian Bot. Soc. 41:604-622. (Abstr. in Rev. Appl. Mycol. 43:17).
3. Atkins, J. G. 1962. Prevalence and distribution of pathogenic races of *Piricularia oryzae* in the U.S. Phytopathology 52: 2. (Abstr.)
4. Atkins, J. G. 1965. Physiologic races of *Piricularia oryzae* in the Western Hemisphere, 9. 243-244 In the Rice Blast Disease. Proc. Symp. at the Int. Rice Research Inst.,

July 1963. Johns Hopkins Press, Baltimore.

5. Chen. M. J. 1966. The carbon and nitrogen utilization ability of *Piricularia oryzae* Cav. in relation to variation of the pathogenicity. Plant Protection Bulletin (Taiwan) 8 (4): 1-11.
6. Chen, Y. S., H. C. Ren, and C. T. Fang. 1964. Study on the carbon and nitrogen nutrition of *Piricularia oryzae* and *Helminthosporium oryzae*. Acta phytopath. Sin. 7: 165-174. (Abstr. in Rev. Appl. Mycol. 44: 395)
7. Chien, C. C., S. Y. Lin, and S. C. Jong. 1963. Studies on the physiologic races of *Piricularia oryzae* Cav. (II). Agr. Res. (Taiwan) 12: 29-39.
8. Chiu, R. J., C. C. Chien, and S. Y. Lin. 1965. Physiologic races of *Piricularia oryzae* in Taiwan, p. 245-255. In The Rice Blast Disease. Proc. Symp. at the Int. Rice Research Inst., July 1963. Johns Hopkins Press, Baltimore.
9. Cochrane, V. W. 1957. Physiolgy of Fungi. John Wiley and Sons, Now York. 246 p.
10. Goto, K. 1960. Progress report of the joint research on the races of the rice blast fungus, *P. oryzae*. Fasc. I, Plant Disease and Insect Pests Forecasting Service Spec. Bull. 5. 89 p.
11. Goto, K. 1965. Physiologic races of *Piricularia oryzae* in Japan, p, 237-242. In The Rice Blast Disease. Proc. Symp. at the Int. Rice Research Inst., July 1963. Johns Hopkins Press, Baltimore.
12. Hung, C. S., C. C. Chien and S. Y. Lin. 1961. Study on the physiologic races of *Piricularia oryzae* Cav. Agri. Res. (Taiwan), 10(1): 27-34.
13. International Rice Research Institute. 1964. Study on the pathogenic races of *Piricularia oryzae* Cav. in the Philippines. p. 131-136. In 1964 Annual Report of the International Rice Research Institute, Los Banos, Laguna, Philippines.
14. International Rice Research Institute. 1964. Nutrition of *Piricularia oryzae* isolates in vitro. p. 136-140. In 1964 Annual Report of the International Rice Research Institute.
15. Kou, T. T., S. C. Woo, and W. H. Wang. 1963. Some physiologic specializations of *Piricularia oryzae* Cav. in Taiwan. Bot. Bull. Acad. Sinica 4: 23-29.
16. Latterell, F. M., M. A. Marchetti, and B. R. Grove. 1954. Co-ordination of effort to establish an international system for race identification in *Piricularia oryzae*, p. 257-274. In The Rice Blast Disease. Proc. Symp. at the Int. Rice Research Inst., July 1963. Johns Hopkins Press, Baltimore.
17. Latterell, F. M., E. C. Tullis and J. W. Collier. 1960. Physiologic races of *Piricularia oryzae* Cav. Plant Dis. Rept. 44: 679-680.
18. Letterell, F. M., E. C. Tullis, R. T. Otten, and A. Gubernik. 1954. Physiologic races of *Piricularia oryzae* Cav. Phytopathology 44: 495. (Abstr.)
19. Leaver, F. W., J. Leal, and C. R. Brewer. 1947. Nutritional studies on *Piricularia oryzae*. J. Bacteriol. 54(4): 401-408.
20. Lee, Y. S. and L. C. Wu. 1967. The nitrogen nutrition of *Piricularia oryzae* Cav. Bot. Bull. Acad. Sinica 8(1): 66-72.
21. Otani, Y 1952 Growth factors and nitrogen sources of *Piricularia oryzae* Cav. Ann

Phytopath. Soc. Japan, 17(1): 9–15.

22. Otani, Y. 1953. Carbon sources of *Piricularia oryzae* Cav. Ann. Phytopath. Soc. Japan 17: 119–120.
23. Otsuka, H., K. Tamari, and N. Ogasawara. 1965. Variability of *Piricularia oryzae* in culture. p. 69–109. In The Rice Blast Disease. Proc. Symp. at the Int. Rice Research Inst., July 1963. Johns Hopkins Press, Baltimore.
24. Suzuki H. 1967. Studies on biologic specialization in *Piricularia oryzae* Cav. Tokyo University of Agriculture and Technology, Tokyo. Japan. 235 p.
25. Tanaka, S. 1965. Nutrition of *Piricularia oryzae* in vitro. p. 23–33. In The Rice Blast Disease. Proc. Symp. at the Int. Rice Research Inst., July 1963. Johns Hopkins Press, Baltimore.
26. Tanaka, S., and H. Katsuki. 1951. Growth factors of the fungus causing rice blast disease. 1. Biotin as the principal growth factor. J. Chem. Soc. (Pure Chem. Sect.), Japan, 72(2/3): 231–233.
27. Tanaka, S., H. Katsuki, and F. Katsuki. 1952. Biochemical studies of the blast disease of rice plants IV. Effect of the different nitrogen sources in the culture media upon the nutritional absorption of the blast disease fungus. Ann. Phytopath. Soc. Japan, 16 (3/4): 103–106.
28. Tochinai, Y. and M. Shimamura. 1932. Studies on the physiological specialization in *Piricularia oryzae* Br et Cav. Ann Phytopath. Soc. Japan, 2: 414–441.
29. Tomizawa, C. and H. Koike. 1953. Studies on the nutritional physiology of the rice blast fungus. Ann. Phytopath. Soc. Japan, 17: 113–118.
30. Tseng, T. C. and L. C. Wu. 1967. The carbon nutrition of *Piricularia oryzae* Cav. Bot. Bull. Acad. Sinica 8(1):73–79.

PART III
INHERITANCE OF RESISTANCE TO DIFFERENT RACES OF *PIRICULARIA ORYZAE*[1]

By
Sung-ching Hsieh[2] ,Ming-hwa Lin[3], Sheng-tian Yen[3] and Hsiao-lan Liang[4]

Introduction

Knowledge of genetics of blast disease resistance is important to rice breeders, but rather little has been accumulated. It is generally believed that rice varieties of the *indica* type are more resistant to the blast disease than the *japonica* varieties (Nakamori 1936, Hashioka 1950, Yamazaki 1959, Hsieh *et. al* 1961,1965). However, this is not consistent with the recent finding of physiological races. It seems not impossible that the resistance to different races of blast fungus be quite differently inherited and genes involved may differ too. Atkins (1965) found two independent genes each conditioning the reaction to a race. Basing on the degree of resistance to seven different races, Yamazaki and kiyosawa (1966) concluded that the dominancy of the resistance genes could vary according to environmental conditions.

The present study was conducted to find out genes controlling the resistances to different blast fungus races occuring in Taiwan, in order to obtain information of basic importance for the resistance breeding in rice.

Materials and methods

Both *japonica* and *indica* varieties of rice in current use by Chien (1963) as the differential hosts to identify physiological races of the blast fungus in Taiwan were mainly used as the cross parents. Some strains from U.S.A., Japan and other countries were also used. In order to avoid hybrid sterility, only such crosses as *japonica* x *japonica*, and *indica* x *indica*. were made. The F_2 plants between these strains grown in an experimental field were recorded for various segregating characters. Then, the F_2 plants and F_3 lines sown in plastic boxes (26x32x12 cm, filled with 6 kg of sandy loam soil and dressed with ammonium sulfate 3.5 gr., calcium superphosphate 2.5 gr., and potassium sulfate 1 gr.,) were tested for blast resistance. About 150 F_2 plants for each cross and 20-50 parental plants were planted side by side for comparison. They were top-dressed twice with 2% ammonium sulfate solution. For the first and second experiments, the materials were divided into two sets for inoculation with races 4 and 13. Inoculation was made at the five-leaf

1) The writers express their hearty thanks to Dr. Atkins of USDA and Dr. H. I. Oka of National Genetics Institute, Japan for various suggestions and criticisms during the experiment. Thanks are also due to Mr. C. C. Chien, Miss S. Y. Lin, Mr. C. L. Chu Plant Pathologists of Taiwan Agricultural Research Institute for furnishing the blast fungus isolates.

2) Senior Agronomist, 3)Junior Agronomists and
4) Research Assistant, respectively.

stage by spraying of spore suspensions onto the leaves and repeated 3-4 times so as to minimize the chance of escaping from infections. The flats were then placed in an inoculation chamber where the moisture (80-90%) and temperature (25-28°C) were controlled. Two days after inoculation the flats were moved out of the inoculation chamber and placed in a green house. The inoculated seedlings were examined and recorded with regard to the lesion types according to the following grading:

Grade	Degree of resistance	Lesion type	Description
O	HR	Immune	No visible sign of infection.
A	R	Brown spot	Small brownish-dark brown lesions or spots.
B	MR	Brown margin	Brownish lesions with a distinct border, which would not increase in size.
C	MS	Grayish white center	Slowly expanding lesions restricted in size.
D	S	Large lesions with dark green center	Large lesions surrounded by brownish bands.
E	HS	Large lesions with grayish center	Lesions are of water soaked appearance, often without well defined border, variable in size,and expanding rapidly.

Note: HR……Highly resistant　　R……Resistant
MR……Moderately resistant　　MS……Moderately susceptible
S……Susceptible　　HS……Highly susceptible

Types O, A and B were considered as resistant (R), while types C, D and E were considered as susceptible (S).

For the third experiment, inoculation was made by an injection methed, injecting spore suspension into the leaf sheath. One week after the injection, the degree of resistance was classified according to lesion types into resistant (R), medium (M) and susceptible (S) groups. The reaction M was considered resistant when the data were analysed. After recording the reaction to the first race, the leaves were clipped off and inoculation with the second race followed four to five days later.

Table 1. List of materials used in the experiment

Variety no.	Variety name	Reaction to races				Remarks
		4	13	22	25	
P109	Taipei 177	R	Sm			Ponlai variety (*japonica*)
P123	Taichung 65	R	S	Sm	S	〃
P130	Taichung 171	R	S	S	R	〃
P137	Kwanfu 401	R	S			〃
P140	Chianung 242	R	S	S	R	〃

P142	Chianan 3	R	S			〃
P151	Kaohsiung 27	R	S			〃
P162	Kwanfu 1	R	S	Sm	R	〃
P167	Pai-kan-tao	R	S	R	S	〃
P169	Chianung-yu 280	R	S	S	R	〃
P173	Taichung line 33	R	S	S	Rm	〃
P174	Taichung 184	R	R	R	R	〃
P175	Taichung 186	R	R	R	R	〃
J302	Nakamura	Rm	Sm			Japanese variety(*japonica*)
J306	Shinriki	R	Sm			〃
J313	Tamanishiki	S	R			〃
J317	Sensho	S	R	R	S	〃
J329	Kanto 51	S	R	R	R	〃
J331	Norin 21	Sm	R	R	R	〃
H25	Motzure-ine	S	R			Dwarf mutant (*japonica*)
H61		Sm	R	S	R	Gene marker from Japan
H106	Dwarf mutant (d_{42})	S	R			Dwarf mutant (*japonica*)
M506	Kung-shen-wu-shen-ken	S	S	R	S	Variety from China-main-land(*japonica*)
S256		S	R			*indica* variety
7102		Sm	Sm	S	S	Gene marker from U. S. A.
IG-65-2		R		Sm	Sm	Isogenic line of Taichung 65
IG-65-3		Rm	S	S	S	〃
C2180		R	R	S	S	Dwarf mutant d_{31}

Results

1.Reaction of F_2 seedlings to races 4 and 13

According to Chien (1963), race 4 was found most frequently in northern (Iran, Taipei) and central (Tungshu, Taichung) parts of Taiwan, while race 13 prevailed throughout the island. In the present study, more severe infection was generally seen when rice seedlings were sprayed with race 13 than with race 4. The infection types that these two races induced in the differential varieties were the same as that reported previously by Chien (1963) in majority of cases; however, in rare reactions in some varieties which are not consistent to the expectation were also observed.

1) Experiment I

The results of observations based on six disease lesion types as mentioned before, were further grouped into resistant and susceptible classes as given in Table 2. In the crosses of Taichung 65 x Norin 21, Taichung line 33 x Sensho, Taichung 171 x Sensho, Chianung 242 x Kanto 51, Chianung 242 x Sensho, the resistant and susceptible plants were segregated

into a 3:1 ratio showing that resistance to races 4 and 13 was each controlled by a single dominant gene.

In some of the crosses, reaction of only one race which gave mostly a 3:1 ratio was observed but reaction to other race was not observed due to the failure of second inoculation, such as Kwanfu 1 x Sensho, Kwanfu 1 x Kanto 51, Taichung 65 x Norin 21, Taichung 171 x Kung-shan-wu-shen-ken, etc. This shows that resistance to races 4 and 13 are each controlled by a single dominant gene which is similar to that reported by Atkins (1965) and Kiyosawa (1965).

Table 2. Reaction of F_2 seedlings to races 4 and 13 (Experiment 1)

Crosses	Races	Parental reactions	Resis-tant	Suscep-tible	Total	Segr. ratio	P
Taichung line 33 x Sensho	4	RxS	195	73	268	3:1	0.3-0.5
	13	SxR	102	36	236	3:1	0.7-0.8
Taichung 171 x Sensho	4	RxS	189	46	235	3:1	0.02-0.05
	13	SxR	129	39	168	3:1	0.5-0.7
Chianung 242 x Kanto 51	4	RxS	98	21	119	3:1	0.1-0.2
	13	SxR	101	20	121	3:1	0.02-0.05
Chianung 242 x Sensho	4	RxS	99	30	129	3:1	0.3-0.5
	13	SxR	74	48	122	3:1	<0.01
Taichung 65 x Norin 21	4	RxSm	42	18	60	3:1	0.3-0.5
	13	SxR	57	14	71	3:1	0.8-0.9
Pai-kan-tao x Kanto 51	13	SxR	77	21	98	3:1	0.3-0.5
Pai-kan-tao x Norin 21	4	RxSm	58	15	73	3:1	0.3-0.5
Kwanfu 1 x Sensho	4	RxS	91	27	118	3:1	0.3-0.5
Kwanfu 1 x Kanto 51	13	SxR	103	31	134	3:1	0.8-0.9
Taichung 171 x Kung-shan-wu- shen-ken	4	RxS	213	74	287	3:1	0.7-0.8
Chianung 242 x Norin 21	4	RxSm	74	28	102	3:1	0.5-0.7
Kwanfu 1 x Norin 21	4	RxSm	96	28	124	3:1	0.5-0.7
Kwanfu 1 x Kung-shan-wu- shen-ken	4	RxS	226	67	293	3:1	0.3-0.5

Table 3. Reaction of F_2 seedlings to races 4 and 13 (Experiment 2)

Crosses	Races	Parental reations	Resis-tant	Suscep-tible	Total	Segr. ratio	P
Tamanishiki x Shinriki	4	SxR	52	23	75	3:1	0.3-0.5
	13	RxSm	64	15	79	3:1	0.3-0.5
Tamanishiki x Kwanfu 401	4	SxR	65	22	87	3:1	0.9
	13	RxS	76	9	85	15:1	0.1-0.2
Shinriki x Sensho	4	RxS	44	29	73	3:1	<0.01
	13	SmxR	54	14	68	3:1	0.6-0.7
Chianan 8 x S 256	4	RxS	38	23	61	3:1	0.02-0.05
	13	SxR	38	19	57	3:1	0.2-0.3
Kaohsiung 27 x Tamanishiki	4	RxS	59	34	93	3:1	0.1-0.2
	13	RxSm	62	14	76	3:1	0.1-0.2
Pai-kan-tao x H 106	4	RxS	59	34	93	3:1	0.7-0.8
	13	SxR	62	14	76	3:1	0.05-0.01
Pai-kan-tao x Nagao H-25	4	RxS	38	18	56	3:1	0.3-0.5
	13	SxR	28	11	39	3:1	0.8-0.9
Taipei 177 x Tamanishiki	13	SmxR	46	22	68	3:1	0.9
Tamanishiki x Nakamura	13	RxSm	42	36	78	9:7	0.3-0.5
Sensho x Nakamura	4	SxR	39	29	68	3:1	<0.01

2) Experiment II

During 1965, different sets of F_2 materials were tested with races 4 and 13, in order to compare the results obtained in the first experiment. The spraying method was again employed for inoculation. The results are given in Table 3. As shown in Table 3, in the crosses of Tamanishiki x Shinriki, Pai-kan-tao x H106, Pai-kan-tao x H-25, Chianan 8 x S-256, a single gene segregation was found when inoculated with both races 4 and 13. This is similar to that of experiment I, showing that a single dominant gene controls resistance to respective race. However, cases of two genes controlling resistance to one race was also found as expressed by a 9:7 or 15:1 ratio. In some cases, the data are not fit too well to the expected ratio. This may be due to chance variation. The result of the second experiment is consistent with that of first experiment.

2. Relation between resistance gene Pi_4 and Pi_{13}

In order to know whether genes resistant to races 4 and 13 are linked or independent from each other, F_2 plants were first inoculated with race 4. After recording of disease reaction, the diseased leaves were clipped off and race 13 were inoculated. By this way, reaction of the same plants to different races can be compared. Strain C-2180 which carries dwarf gene d_{31} is resistant to both races 4 and 13, while purple leafed gene marker strain 7102 is moderately susceptible to both races 4 and 13. In the F_2 of C-1280×7102, single gene segregation indicated by a 3:1 ratio was seen when inoculated with both races. As shown in Table 4, the blast resistance gene named Pi_4 and Pi_{13} were found to be independent from each other after the chi-square test. Same is true in the cross of IG-65-2×7102 as shown in Tables 4 and 5.

Table 4. Seedling reaction of F_2 plants of C-2180 x 7102 to races 4 and 15

		Reaction to race 4			P for a 3:1 ratio
		R	S	Total	
Reaction to race 12	R	67	15	82	0.90-0.95
	S	20	7	27	
	Total	87	22	100	
P for a 3:1 ratio		0.20-0.30			

P for independence was 0.30-0.50

Table 5. Seedling reaction of F_2 plants of IG-65-2x 7102 to races 4 and 13

		Reaction to race 4			P for a 3:1 ratio
		R	S	Total	
Reaction to race 13	R	82	32	114	0.20-0.30
	S	38	9	47	
	Total	120	41	161	
P for a 3:1 ratio		0.80-0.90			

P for independence was 0.20-0.30.

Table 6. Reaction of F_2 Seedlings to races 22 and 25

Crosses	Races	Parental reactions	Resistant	Susceptible	Total	Segr. ratio	P
Chianung 242 x Norin 21	22	SxR	170	59	229	3:1	0.7-0.8
Sensho x Taichung 171	22	RxS	45	18	63	3:1	0.5-0.7

Sensho x Kung-shan-wu-shen-ken	22	RxS	117	28	145	3:1	0.1-0.2
Taichung 184 x Pai-kan-tao	25	RxS	166	51	217	3:1	0.5-0.7
Taichung 184 x Kung-shan-wu-shen-ken	25	RxS	217	65	282	3:1	0.3-0.5
Taichung 186 x Taichung 65	25	RxS	217	58	275	3:1	0.1-0.2
Taichung 186 x Pai-kan-tao	25	RxS	203	72	275	3:1	0.5-0.7
Sensho x Taichung line 33	25	SxR	180	66	246	3:1	0.5-0.7

3. Reaction of F_3 plants to races 22 and 25

As shown in Table 1, the parental strains Pai-kan-tao is resistant to race 22 and susceptible to race 25, Chianung-yu 280 is susceptible to race 22 and resistant to race 25. The reaction to the two races of their F_2 plants are in Table 7. The F_2 ratios both gave a good fit to the 3:1 ratio, indicating that the resistance to the two races were each controlled by a dominant gene. The data in the Table 7 show that the two genes controlling resistance to the two races are independent of each other. They are named Pi_{22} and Pi_{25} respectively. The F_2 plants between Chianung-yu 280 and Sensho also gave the same pattern of segregation as above as shown in Table 8.

Kun-shan-wu-shen-ken is resistant to race 22 but susceptible to race 25. In its cross with Chianung-yu 280, the F_2 ratios also gave a good fit to a 3:1 ratio. P for independence test between Pi_{22} and Pi_{25} was 0.30 to 0.50 showing no correlation between these two genes, as shown in Table 9. Taichung line 33 is highly susceptible to race 22, while Taichung 65 is moderately susceptible in this experiment. In the F_2 of cross between highly susceptible and moderately susceptible to race 22, the moderately resistant and susceptible plants were segregated into a 3:1 ratio. The independent relation between genes Pi_{22} and Pi_{25} was again found in the cross between Taichung line 33 x Taichung 65 as shown in Table 10. The same pattern of inheritance regarding the resistance to race 22 and 25 were found in the crosses of Taichung line 35 x Norin 21 and Taichung 171 x Taichung 65 as given in Tables 11 and 12.

Table 7. Seedling reaction of F_2 plants of Chianung-yu 280 x Pai-kan-tao to races 22 and 25

		Reaction to race 22			P for a 3:1 ratio
		R	S	Total	
Reaction to race 25	R	151	48	199	0.5-0.7
	S	47	15	62	
	Tatal	198	63	261	
P for a 3:1 ratio		0.70-0.80			

P for independence was 0.99-1.00

Table 8. Seedling reaction of F_2 plants of Chianung-yu 280 x Sensho to races 22 and 25

		Reaction to race 22			P for a 3:1 ratio
		R	S	Total	
Reaction to race 25	R	125	37	162	0.99-1.00
	S	39	15	54	
	Total	164	52	216	
P for a 3:1 ratio		0.70-0.80			

P for independence was 0.30-0.50

Table 9. Seedling reaction of F_2 plants of Chianung-yu 280 x Kung-shan-wu-shen-ken to races 22 and 25

		Reaction to race 22			P for a 3:1 ratio
		R	S	Total	
Reaction to race 25	R	150	40	190	0.80-0.90
	S	47	16	63	
	Total	197	56	253	
P for a 3:1 ratio		0.20-0.30			

P for independence was 0.30-0.50

Table 10. Seedling reaction of F_2 plants of Taichung line 33 x Taichung 65 to races 22 and 25

		Reaction to race 22			P for a 3:1 ratio
		R	S	Total	
Reaction to race 25	R	170	55	225	0.30-0.50
	S	55	12	67	
	Total	225	67	292	
P for a 3:1 ratio		0.30-0.50			

P for independence was 0.20-0.30

Table 11. Seedling reaction of F_2 plants of Taichung line 33 x Norin 21 to races 22 and 25

		Reaction to race 22			P for a 3:1 ratio
		R	S	Total	
Reaction to race 25	R	158	48	206	0.50-0.70
	S	51	24	75	
	Total	209	72	281	
P for a 3:1 ratio		0.70-0.80			

P for independence was 0.10-0.20

Table 12. Seedling reaction of F_2 plants of Taichung 171 x Taichung 65 to races 22 and 25

		Reaction to race 22			P for a 3:1 ratio
		R	S	Total	
Reaction to race 25	R	151	40	191	0.10-0.20
	S	36	14	50	
	Total	187	54	241	
P for a 3:1 ratio		0.30-0.50			

P for independence was 0.20-0.30

4. Reaction of F_3 plants to races 4 and 22

Pai-kan-tao is highly resistant to race 4, while H-61 is susceptible. The F_3 lines of their hybrid could be divided into resistant, segregating and susceptible classes, giving a good fit to the 1:2:1 ratio (Table 13). Same is true in the crosses of IG-65-2 x P167 and IG-65-3 x P167. Presence or absence of linkage between Pi_4 and Pi_{22} was not tested owing to a failure of inoculation with race 22.

Table 13. Reaction of F_3 seedlings to races 4 and 22

Cross	race	Observed no. of lines				Expected ratio	X^2	P
		R	Seg.	S	Total			
P167		9						
P167xH-61	4	70	136	64	270	1:2:1	0.2815	0.8-0.9
H-61				10				

IG-65-2				14				
IG-65-2xP167	22	52	119	57	228	1:2:1	0.6529	0.7-0.8
P167		12						
IG-65-3				14				
IG-65-3xP167	22	73	140	60	281	1:2:1	0.1815	0.9-0.95
P167		12						

5. Genotypes of blast resistance in parental varieties

The segregation ratios of resistance to races 4 and 13, 22 and 25 in the above mentioned experiment are summarized in Tables 14 and 15. The blank in these tables still needed to be filled out by the further test. The genotypes for resistance genes of the ten parental strains may then be presumed as follows:

Pai-kan-tao ·····Pi_4; pi_{13}; Pi_{22}; pi_{25}
Sensho ·····Pi_{13}; Pi_{22}
Chianung-yu 280. ···Pi_4; pi_{22}; Pi_{25}
IG-65-2 ·····Pi_4; pi_{13}; pi_{22}
IG-65-3 ·····Pi_4; pi_{13}; pi_{22}
H-106·····pi_4
C-2180 ·····Pi_4; Pi_{13}
Taichung line 33·····Pi_4; pi_{22}; Pi_{25}
Taichung 65 ·····pi_{22}; pi_{25}
Kanto 51 ·····pi_4; Pi_{13}; Pi_{22}; Pi_{25}

Varieties identified to carry blast resistance genes Pi_4, Pi_{13}, Pi_{22} and Pi_{25} are given in Table 16.

Table 14. Segregation ratios of resistance and susceptible to races 4 and 13 of *Piricularia Oryzae*

	Kanto 51		Pai-kan-tao		Tamanishiki		Sensho		S-256	
	race 4	race 13	race 4	race 13	race 4	race 13	race 4	race 13	race 4	race 13
Kwanfu 1		3:1					3:1			
Kwanfu 401	3:1	3:1			3:1	15:1				
Chianung 242							3:1	3:1		
Chianung-yu 280			3:1				3:1			
Chianan 8					3:1	3:1			3:1	3:1
Kaohsiung 27					3:1	3:1				
Taichung line 33							3:1	3:1		
Taichung 171							3:1	3:1		
IG-65-2			3:1							
IG-65-3			3:1							

Shinriki	3:1				3:1	3:1	3:1	3:1		
Nakamura						9:7	3:1			
Kanto 51				3:1						
Norin 21			3:1							
H-25			3:1	3:1						
H-106			3:1	3:1						

Table 15. Segregation ratio of resistance and susceptible to races 22 and 25

	Taichung 65		Taichung 184	Taichung 186	Chianung-yu 280		Sensho		Norin 21	
	race 22	race 25	race 25	race 25	race 22	race 25	race 22	race 25	race 22	race 25
Pai-kan-tao	3:1	3:1	3:1	3:1	3:1	3:1		3:1		
Taichung line 33	3:1	3:1						3:1	3:1	3:1
Taichung 171	3:1	3:1					3:1	3:1		
Taichung 186		3:1								
Kung-shan-wu-shen-ken	3:1	3:1	3:1	3:1	3:1	3:1	3:1			
Sensho					3:1	3:1			3:1	3:1

Table 16. Varieties identified to carry blast resistance genes Pi_4, Pi_{13}, Pi_{22}, Pi_{25}

Blast resistance genes	Variety name
Pi_4	Taichung 65, Taichung 171, Taichung lines 33, Kwanfu 1, Kwanfu 401, Chianan 8, Chianung 242, Kaohsiung 27, Nakamura, Shinriki, Pai-kan-tao, C-2180.
Pi_{13}	Taichung 171, Taichung 184, Taichung 186, IG-65-2, Tamanishiki, Sensho Kanto 51, Norin 21, H-106, C-2180
Pi_{22}	Pai-kan-tao, Kanto 51, Norin 21
Pi_{25}	Taichung 171, Taichung 184, Taichung 186, Taichung line 33, Kwanfu 1, Kanto 51, Chianung-yu 280

6. Relation between leaf- and neck-blast resistances

How the resistance of leaf and that of panicle neck are correlated has been a problem of general interest. As shown in Table 17, 81% of strains tested showed positive correlations between leaf and neck blast resistances, while the remaining 19% showed no correlations. Question then arises as to whether or not the leaf and neck blast resistances are controlled by the same genes. Hashioka (1950) reported that some varieties, the number of the infected ears, has no correlation with the susceptibility of the leaves. The writer (Hsieh 1965) estimated phenotypic and genetic correlations between leaf and neck blast resistances in F_2 populations. He found that though phenotypic correlations differed according to crosses, genetic correlations were in most crosses high (rg=0.3-0.9). This suggests that the same genes control both leaf and neck resistances. The reason for the absence of the cor-

relation in certain crosses seems to need further investigations.

Table 17. Number of strains classified according to leaf-and neck-blast resistances

Relation between leaf and neck blast resistances	Locations							
	Taipei	Taichung	Chiayi	Kaohsiung	Taichung	Total	%	
L. N.	65	29	60	73	39	265	43.5	81
l. n.	38	68	37	35	50	228	37.5	
L. n.	9	28	4	10	10	61	10.0	19
l. N.	12	0	23	6	14	55	9.0	

Note: L.N.·····Resistant to both leaf and neck blasts
l. n.·····Susceptible to both leaf and neck blasts
L. n.·····Resistant to leaf blast but susceptible to neck blast
l. N·····Susceptible to leaf blast but resistant to neck blast

7. Test of linkage between Pi_4, Pi_{22} and other maker genes

One of the parental strain H-61 carries C^{BP}, *A p* for apiculus coloration. *Bf* for brown or dark furrows and d_2 for its dwarf stature. Pai-kan-tao has *C a P* ps_1 ps_2 *Bf* for the above characters and ligulelessness gene *lg*. From the cross of these two strains, Pi_4 as well as Pi_{22} were found to be independent of all the above mentioned genes. In the cross of pai-kan- tao with IG-65-2 or IG-65-3 carrying the phenol reaction gene, *Ph* and/or a red pericarp genes *Rc*, *Rd*, Pi_{22} was found to be independent of *Ph* and *Rc*.

Table 18. Test of independence between blast resistance genes and those for other characters

Crosses	Blast resistance genes	Marker genes	Combined characters					P
			AB	Ab	aB	ab	Total	
P167× H-61	Pi_4	*A*;*P*(Apiculus color)	110	96	35	29	270	0.80-0.90
P167× H-61	Pi_4	Ps_1;Ps_2 (Stigma color)	41	69	13	32	145	0.99-1.00
P167× H-61	Pi_4	*Bf*; I-Bf (Brown furrow for lemma and palea)	23	86	6	30	145	0.50-0.70
P167× H-61	Pi_4	d_2 (Ebisu dwarf)	155	51	51	13	270	0.30-0.50
IG-65-2× P167	Pi_{22}	*Rc*	115	56	40	17	228	0.50-0.70
IG-65-2× P167	Pi_{22}	*Ph* (Phenol reaction)	133	37	47	9	226	0.30-0.50
IG-65-3× P167	Pi_{22}	*Ph*	153	60	49	18	281	0.80-0.90

Table 19. Test of independence between ligulelessness gene *lg* and blast resistance genes.

Crosses	Blast resistance genes	Ligulelessness genes	Combined characters					P
			AB	Ab	aB	ab	Total	
P167×H-61	P_4	*lg*	143	58	46	18	270	0.99–1.00
P167×J331	Pi_4	*lg*	36	13	16	5	70	0.99
P167×P123	Pi_{13}	*lg*	121	23	40	13	197	0.10–0.20
P167×J331	Pi_{13}	*lg*	29	11	33	4	77	0.30–0.50
P167×J329	Pi_{13}	*lg*	51	21	16	5	93	0.95–0.98
IG-65-2×P167	Pi_{22}	*lg*	138	33	49	8	223	0.30–0.50
IG-65-3×P167	Pi_{22}	*lg*	164	49	57	11	281	0.20–0.30
P169×P167	Pi_{22}	*lg*	148	50	45	18	261	0.50–0.70
P169×P167	Pi_{25}	*lg*	148	53	48	16	265	0.80–0.90
P174×P167	Pi_{25}	*lg*	124	42	37	14	217	0.70–0.80

In our previous experiment using natural infection (Hsieh *et. al.* 1965), *lg* was linked with an unidentified resistance gene. Also by natural infection, Oka and Lin (1957), reported a linkage between *Ph* and a resistance gene. In view of the above results of linkage test, Pi_4,Pi_{13},Pi_{22} and Pi_{25} may not be synonymous with these unidentified resistant genes.

8.Test of breeding materials with different races of blast fungus

In the light of recent findings on pathogenic races of the blast fungus, breeders in Taiwan are realizing the importance to take this problems into consideration. Test of the breeding materials with races of the fungus were made as the first step in this direction. 50 strains from Taiwan, Japan, U.S.A., India and philippines were tested with 27 races of the blast fungus. Races 12, 13 and 16 are of the most pathogenic ones among the 27. There was a trend for most introduced varieties, either *japonica* or *indica* type, to show resistant reaction to a majority of the races tested. In contrast, varieties which have long been grown in Taiwan showed a wide range of variation in the susceptibility to Taiwanese races. This may be of interest to notice.

In Table 20. The varieties were classified according to the number of races to which they showed resistance.

As shown in Table 18, Taipei 306, Taipei 308, and Kaohsiung 24 were resistant to only one of eight races tested. The U.S. variety Caloro was resistant to only two of ten races. Japanese varieties Pi no. 1, Ginga, Homare-nishiki showed resistances to 9 races, while Chokoto, Yakeiko, Ishikarishiroke were resistant to all the 10 races tested. Zenith, Rexaro (from U.S.A.), Tadukan, Dular (from India), Wag-wag (from Philippines) also showed resistances to all the 10 races tested. Taichung 65 a representative of Ponlai variety in Taiwan was resistant to 10 races but was susceptible to 17 races. Taichung native 1 (*indica*), showed resistance to 7 of 8 races tested.

Taichung line 33, Chianung-yu 280, Norin 21 and Kanto 51 were the most resistant ones showing resistances to 18 of 27 races tested. The more resistant genes a variety carries, the more it would be desirable. Such varieties may be obtained by combining many resistance genes by hybridization. Varieties carrying many blast resistance genes, such as Taichung lines 33, Kanto 51, Norin 21, Chianung-yu 280 and Pai-kan-tao, may be used as the cross parents.

Even though gathering of all resistant genes in a veriety is not possible, it is not difficult to combine in a variety, genes controlling the resistance to two or three races predominating in a region.

Table 20. Varieties grouped according to the resistance to different races of the blast fungus

Number of races of which resistance is found	Variety name
1	Taipei 306*, Taipei 308*, Kaohsiung 24*,
2	Taipei 177*, Hsinchu 56*, Tainan 1*, Chianan 8*, Kaohsiung10*, Kaohsiung 27*, Kaohsiung 64*, Kaohsiung 122*, Tainan 1*, Caloro**
3	Taichung 178*
4	Tainan 3*
5	Hsinchu 61*, Ai-chio-chien*, Natala
6	Norin 20*, Aichi-asahi*
7	Taichung native 1*, Kung-shan-wu-shen-ken
8	Taichung 184*, Taichung 185*, Norin 22*, Lacrose**
9	Pi no. 1*, Ginga**, Homare-nishiki**
10	Taichung 65, Chokoto**, Yakeiko**, Ishikari-shireke**
11	Zenith**, Rexaro**, Tadukan**, Wag-wag**, Dular**, Raminad Str. 3**, CI 5309**
12	Taichung-ti-chio-wu-chien
13	Kao-chio-liu-chao, Kaohsiung-ta-li-chen-yu
14	Chianung 242, Cutsugucul
15	Kwang-fu 1
16	Sensho
17	Taichung 171, Pai-kan-tao
18	Taichung line 33, Chianung-yu 280, Norin 21, Kanto 51.

Note:
1. *...Tested with eight races i. e. nos. 1,5,12,13,16,17,24 and 26.
2. **...Tested with ten races i. e. nos. 3,5,11,13,14,16,17,22 and 26.
3. .....Unmarked ones were tested with all 27 races.

Discussion

As mentioned in the introduction, recent genetic studies of blast diseases resistance using identified fungus races proved that the resistance to a particular race was controlled by a dominant gene (Atkins 1965, Kiyosawa 1965). The results we obtained in this study

are consistent with this. We found the four resistance genes Pi_4, Pi_{13}, Pi_{22} and Pi_{25}, which seemed to be independent of one another in some cases. The above-named workers also reported independency of resistance genes they found. A number of genes controlling resistance to different fungus races might be distributed in different chromosomes. If more linkage experiments are made, their linkage relations with marker genes may be worked out.

It seems that when a particular fungus races is used in an experimental condition, the "gene-to-gene" hypothesis of Flor (1955) generally applies. Segregation of two or more genes reported by earlier workers Nakamori 1936; Hashioka 1950; Yamazaki 1959; Hsieh *et. al.* 1961, 1965 might be due to the use of a mixture of different races or natural infection. However, breeders experience that "field resistance" can not always be explained well by the results of controlled experiments, How the resistance genes work in different genetic backgrounds and in different environments needs further investigation.

It is concluded that blast resistance is simply inherited character. Resistance of a variety to different races is due primarily to a single gene though it is possible minor or modifying genes also influence to degree of resistance. More experiments are to be made in the future to look into this problem.

Summary

1) The F_2 and F_3 hybrids between 28 *japonica* rice strains were tested for blast disease resistance by using four different races, 4, 13, 22 and 25. Spore suspension was inoculated with rice seedlings at the five-leaf stage by using both spraying and injection methods.

2) The resistance to the four races were found to be each controlled by a resistant gene. The four resistance genes found, Pi_4, Pi_{13}, Pi_{22} and Pi_{25} were independent of one another in some cases. Also they were not linked with marker genes involved in the crosses, *A,P, Ps, lg, Bf, I-Bf,* d_2, *Rc* and *Ph.*

3) The resistances to leaf and neck blasts widely varied due to variety, year, crop season and location. In 81% of strains so far tested, leaf and neck resistances were correlated, and appeared to be controlled by the same genes. In the remaining 19%, the leaf and neck resistances were not correlated. Whether or not this is due to the occurrence of different races remains unknown.

4) 50 rice strains from Taiwan, Japan, U.S.A., India and Philippines were tested with the 27 races of blast fungus. Races 12, 13 and 16 are the most pathogenic ones among them. Newly introduced varieties, either *japonica* or *indica* type, tended to be resistant to a majority of the races tested. Taichung line 33, Chiauung-yu 280, Norin 21 and Kanto 51 were the most resistant ones, showing resistances to 18 of the 27 races tested. These varieties were considered to be desirable materials for the blast resistance breeding program in Taiwan.

Literature Cited

1. Atkins, J. G and T. H. Johnston, 1965: Inheritance in rice reaction to races 1 and 6 of *Piricularia Oryzae.* Phytopathology 55(9):993-995.
2. Chien, C. C., S. Y. Lin and S. C. Jong, 1963: Studies on the physiologic races of

Piricularia Oryzae cav. II. Jour. Taiwan, Agr. Res. 12(3):29-39.

3. Chang, T. M and S. C. Hsieh, 1965: Further studies on the inheritance of resistance to leaf and neck blast diseases in rice. Genic analysis in rice VI. Jour. Taiwan. Agr. Res. 14(4):1-9.
4. Chang, T. T., 1961: Recent Advances in rice breeding in Taiwan. Chinese-American Joint Comission on Rural Reconstruction. PID-C-165.
5. Flor, H. H., 1955: Host-parasite interaction in flax rust; its genetics and other implications. Phytopathology 45:680-685.
6. Hsieh, S. C., 1965: Genic analysis in rice V. Genes for the resistance to leaf and neck blast diseases in rice. Bot. Bull. of Acadimia Sinica. 6(1):48-60.
7. Hsieh, S. C., M. H. Lin and H. L. Liang, 1967: Genetic Analysis in Rice. VIII. Inheritance of Resistance to races 4, 22 and 25 of *Piricularia Oryzae*, Bot. Bull. Acadimia Sinica 8:255-260
8. Hashioka, Y., 1950: Studies on the mechanism of prevalence of the rice blast disease in Tropics. Taiwan. Agr. Res. Inst. Technical Bulletin No. 8.
9. Kiyosawa, S., 1966: Studies on inheritance of resistance of rice varieties to blast. 2. genetic relationship between the blast resistance and other characters in the rice varieties, Japan Jour. Breeding 16(2):15-23.
10. Ou, S. H. and K. Lin, 1959: Preliminary report on rice breeding for blast disease resistance in Taiwan. Chinese-American Joint Commission on Rural Reconstruction. Plant Industry Series. No. 15:23-32.
11. Tsai, K. H. and H. I. Oka, 1965: Genetic studies of yielding capacity and adaptability in crop plant. 1. Characters of isogenic lines in rice. Bot. Bull. of Academia Sinica. 6(1):19-31.
12. Yamasaki, Y and Kiyosawa, 1966: Studies on inheritance of resistance of rice varieties to blast. 1. Inheritance of resistance of Japanese varieties to several strains of the fungus. Bull. Nat. Inst. of Agr. Sci. Japan. Series D. No. 14:39-69.
13. Nursery Trials on rice varietal Reactions to Blast, A Summary Report for 1956-1964. Taiwan Provincial Dept. of Agriculture and Forestry.

NEED FOR ADDITIONAL RESEARCH

Future plan of breeding for resistant varieties

1) Breeding of varieties having more than two major resistant genes.

As the data in the present studies show, Taipei 306, Taipei 308, and kaohsiung 24 are resistant only to race 1. They are presumed to carry one resistant gene, Pi_1. If other races than no. 1 predominant, these varieties may lose their resistance. Varieties with two or more resistant genes, may not lose resistance so easily. Such varieties must be established. In practice, however, the more the resistance genes to be combined, the more difficult would be the work, and some resistant genes may be linked with other genes controlling undesirable

characters. Cross parents may be selected from varieties listed in Table 20.

2) Breeding of isogenic lines each carrying a major resistance gene.

Breeding of varieties carrying a major gene is relatively easy. Varieties adaptive to different regions may be used as the recurrent parents of back-crosses for obtaining isogenic lines with certain resistant genes. Tsai and Oka (1965) developed isogenic lines of Taichung 65 carrying genes for early maturity, positive phenol reaction, glutinous endosperm etc, by back-crossing. Since the degree of blast resistance of a strain widely varies due to locations and yeârs, environmental modifications must be carefully observed for performing this work. If many isogenic lines become available, a few selections of them may be grown according to the fungus races predominating in a given region. Recording and forecasting of prevalent races should be made every year in each locality. pathogenic mutations of the races if frequently may make this effort fruitless, however.

3) Mixture of various isogenic lines to make up a variety

Various isogenic lines of the same variety may be mixed to form a "multi-line". The variety, kind and number of isogenic lines to be mixed must be determined according to the prevalent races in a given region. Such a "multi-line" veriety may be usefull especially when various races spontaneously occurred in a region. Though the breeding of many isogenic lines needs a long time and much labor, this may be attempted in the future.

4) Application of field resistance

Varieties truely resistance to a race or races are not always completely resistant in the field where the given race or races prevail. The mechanism of "field resistance" seems to remain unknown. Minor genes may possibly be concerned. It is important to select field resistance together with true resistance. It seems that a breeding program should be conducted under both controlled and natural conditions.